

Contribution of Adventitial Fibroblasts to Neointima Formation and Vascular Remodeling

From Innocent Bystander to Active Participant

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Abstract—The adventitial layer surrounding the blood vessels has long been exclusively considered a supporting tissue the main function of which is to provide adequate nourishment to the muscle layers of tunica media. Although functionally interconnected, the adventitial and medial layers are structurally interfaced at the external elastic lamina level, clearly distinguishable at the maturational phase of vascular morphogenesis. Over the last few years the “passive” role that the adventitia seemed to play in experimental and spontaneous vascular pathologies involving proliferation, migration, differentiation, and apoptosis of vascular smooth muscle cells (VSMCs) has been questioned. It has been demonstrated that fibroblasts from the adventitia display an important partnership with the resident medial VSMCs in terms of phenotypic conversion, proliferation, apoptotic, and migratory properties the result of which is neointima formation and vascular remodeling. This article is an attempt at reviewing the major themes and more recent findings dealing with the phenotypic conversion process that leads adventitial “passive” (static) fibroblasts to become “activated” (mobile) myofibroblasts. This event shows some facets in common with vascular morphogenesis, ie, the process of recruitment, incorporation, and phenotypic conversion of cells surrounding the primitive endothelial tube in the definitive vessel wall. We hypothesize that during the response to vascular injuries in the adult, “activation” of adventitial fibroblasts is, at least in part, reminiscent of a developmental program that also invests, although with distinct spatiotemporal features, medial VSMCs. (*Circ Res.* 2001;89:1111-1121.)

Key Words: adventitia ■ vascular smooth muscle cells ■ myofibroblasts
■ neointima formation and vascular remodeling

The arterial wall represents a highly plastic three-dimensional structure with a unique adaptive capacity to face changes in blood pressure, flow, and shear stress taking place during development and in some vascular pathologies.¹⁻⁴ This functional behavior is due to the peculiar assembling of cellular and extracellular components into the following three distinct tunicae or layers: intima, media, and adventitia.³ This tissue compartmentalization inherently gives rise to some structural-functional interfaces of which the integrity allows for the maintenance of arterial wall homeostasis.⁴ Pathological stimuli can target all wall layers and interfaces simultaneously or one layer/interface primarily.⁵ The response to injury may be initially localized to a specific layer, and later it can invest other layers depending on time, severity of the lesion, responsiveness, and functional connectivity among the wall tissues.^{1,2,4-6} At both sides of the arterial wall, a repair program is activated that includes neovascularization, infiltration of inflammatory cells, local

cell proliferation, differentiation, and apoptosis along with extracellular matrix (ECM) deposition.^{1,2,4-7} This repair process bears, to some extent, the possibility of a positive vascular wall remodeling, ie, the maintenance of an acceptable function in the presence of structural change.⁸

The role of adventitia in the context of providing cells and molecules with the capacity to influence neointima formation and vascular remodeling has recently received considerable attention.⁹⁻¹³ In particular, it has been observed that during response to injury, resident cells can be reprogrammed to have different structural and functional behavior. For example, adventitial fibroblasts in experimental models of severe and mild balloon injury⁹⁻¹² can be phenotypically converted into smooth muscle (SM)-like cells, the myofibroblasts. Conversely, medial vascular SM cells (VSMCs), when activated in the course of neointima formation or in atherogenesis, display structural and functional features common to immature, developmentally related cells.^{1,4,6} Thus, in re-

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sponse to injury both the adventitial and the medial layers retrieve a “default” cell that probably best suits the repair process. Because this repair cell phenotype is also found during vascular morphogenesis, we can hypothesize that the mechanism of response to injury in the adult wall may be, at least in part, reminiscent of a developmental process.

The following key questions will be tackled in this review: (1) Do neointimal cells arise from adventitia and, if so, which is their relative contribution to neointima formation? (2) In autologous vein-to-artery graft and transplant arteriosclerosis (TA), do the neointima derive from the host/donor adventitia, the host/donor medial layer, or the circulation? To address these issues, the review will be divided into the following sections: (1) the formation of the tunica adventitia; (2) a synopsis of structural-functional features of fibroblasts and myofibroblasts; and (3) the role of the adventitia in the response to injury, in autologous vein-to-artery graft and TA.

Formation of the Tunica Adventitia

Below is a brief description of vascular morphogenesis that highlights some dynamic features particularly relevant to the pathophysiological hypothesis outlined above. As for the topics not covered in this review, eg, microcirculation, pericyte origin, factors involved in cell recruitment, and vascular wall maturation, there are excellent reviews that will furnish the reader with details not included in the present analysis.^{14,15}

The formation of the tunica adventitia is a progressive phenomenon the cellular and molecular events of which are related to nature, size, and regionalized differences of the vessels. The primitive vascular network forms by a process called vasculogenesis, in which endothelial cells (ECs) proliferate and coalesce to form a primitive vascular bed that provides blood flow and oxygenation to the embryo.^{14,15} Recruitment of mural cells to the endothelial tube is the second major step in vasculogenesis¹⁶ and is critical in determining either the stabilization or the regression of the early blood vessels. There are several possible sources of mural cell progenitors (Figure 1A). These cells can be recruited either from the local mesenchyme or from the distal mesenchyme, which in turn can be of neural crest (great vessels connected to the heart) or proepicardial origin (coronary vessels).¹⁷ Furthermore, it has been shown that ECs of the chicken dorsal aorta can transdifferentiate into VSMCs in the developing vessel¹⁸ and that hemopoietically derived cells may participate in vascular morphogenesis via formation of ECs or VSMCs depending on vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF)-BB availability.¹⁹

The cells with the first sign of VSMC commitment are the vascular myoblasts that can be identified from their expression of SM α -actin (Figure 1A and Table 1). Appearance of these cells is not synchronous in all of the vessels and is also regionalized within the same vessel. Gathering of vascular myoblasts around the endothelium precedes lumenization, but transcriptional activation of the SM α -actin gene follows formation of the endothelial tube.³ This evidence supports the idea that factors emanating from the endothelium, possibly via local exposure to shear stress, mechanic stretch, flow

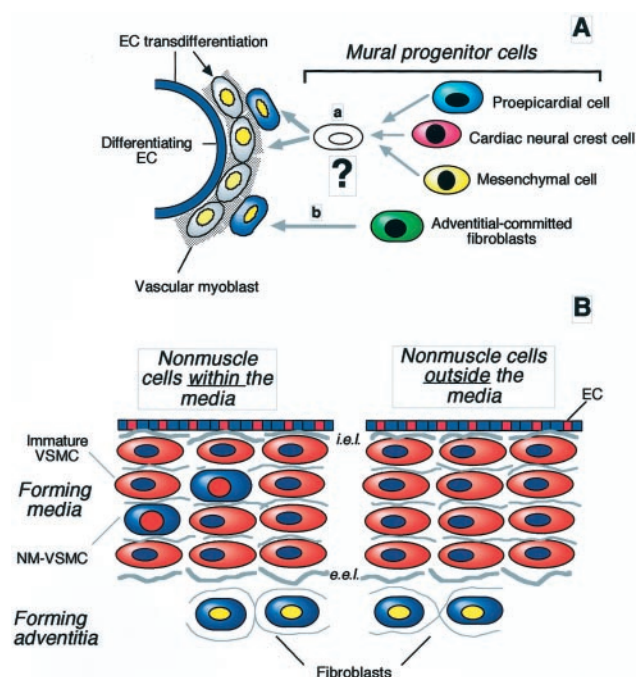


Figure 1. Diagram showing the two major steps during arterial morphogenesis, from the recruitment and incorporation of mural progenitor cells (A) to the maturing vascular wall with medial and adventitial tissue diversification (B). A, Illustration of existence of a common (a) or a distinct (b) progenitor cell for adventitial fibroblasts and vascular myoblasts. B, Two forming media, one composed of immature VSMCs alone (right) and the other composed of immature VSMCs and NM cells (left). In some species, immature VSMCs persist in the media of adult vessels.^{4,21} Note that fibroblasts and NM-VSMCs are differently identified despite their antigenic properties being identical (see also comments regarding Table 1). i.e.l. indicates internal elastic lamina; e.e.l., external elastic lamina.

pattern, local oxygen, and nutrient demand, induce the recruitment and the differentiation of vascular myoblasts.¹⁶

Progressive expression of SM α -actin, nonmuscle (NM) and SM myosin isoforms,^{4,20} identifies, in the prenatal and postnatal period, the immature and the differentiated VSMCs, respectively (Table 1). These two cell types each show a distinct profile with respect to proliferation and synthesis of ECM proteins. Differentiated VSMCs display a marked phenotypic plasticity when grown in vitro and can dedifferentiate (or phenotypically modulate) to cells with growth, differentiation, and synthetic properties similar to those of the immature VSMCs and the myofibroblasts (see below).⁴

In the adult, the following two major VSMC subsets can be identified in the medial layer (Table 1): the differentiated VSMCs and the NM-VSMCs. The NM-VSMC subset has a very limited repertoire of SM markers, and its contribution to the media differs greatly among the vessels and in different species (Figure 1B).^{1,2,4,6,21} In vitro, clonal studies have confirmed the existence of the following two morphologically and functionally distinct VSMCs: the spindle-shaped and the epithelioid VSMCs.^{22,23} The spindle cells are shown to be well differentiated, with a slight tendency to migrate or proliferate, whereas the epithelioid cells are less differentiated, can grow in the absence of serum, and have a good migratory ability. It is not completely clear which correlation

TABLE 1. Immunocytochemical Identification of Major Cell Types in Developing and Adult Large Blood Vessels and In Experimental Models of Wall Injury

Name	Identification				
	Vimentin	NM Myosins	α -SM Actin	SM Myosins	Smoothelin
Nonmuscle cells					
Fibroblasts/NM-VSMCs* ^{1,2,4,32,33}	+	+	–	–	–
Cells showing a variable assortment of nonmuscle smooth muscle antigenic properties					
Vascular myoblasts† ^{3,14–16}	+	+	+	–	–
Myofibroblasts‡ ^{11,32}	+	+	+	–	–
Smooth muscle cells					
Immature VSMCs ^{1,4,20,102}	+	MyHC-B ⁺ MyHC-Apla1 ^{+,2+}	+	SM1 ⁺ , SM2 [–]	–
Differentiated VSMCs ^{1,4,20,59}	+	MyHC-B ⁺ MyHC-Apla1 ^{–,2+}	+	SM1 ⁺ , SM2 ⁺	+

MyHC indicates myosin heavy chain; MyHC-B, B-type MyHC isoform; MyHC-Apla1,2, platelet-type 1 and 2 MyHC myosin isoforms; and SM1 and SM2, smooth muscle-type 1 and 2 MyHC isoforms.

*NM cells are identified as fibroblasts or NM-VSMCs on the basis of their localization in the adventitia or media, respectively.

†This cell expresses first SM α -actin (once is able to acquire a positional control, ie, after contact with the endothelium) and then an α -actinin isoform and SM22 α .^{1,3}

‡Myofibroblasts encompass a heterogeneous cell population. In response to injury, the predominant cell variant present in adventitia is the VA isotype.³²

exists between the VSMC phenotypes seen in vitro and in vivo and whether distinct VSMC lineages exist in the vascular wall.^{1,5,22,23} The presence of distinct VSMC clones, however, is not incompatible with a cell phenotypic switch in response to various stimuli.²⁴ This implies an important role for environmental cues in inducing phenotypic plasticity. Cell targeting and cell cloning experiments will be vital in addressing these important issues.

The predominant cell in the tunica adventitia is the fibroblast. Because of the lack of specific markers, it is still unclear whether VSMCs and fibroblasts arise from distinct or common progenitors (Figure 1A). In the developing coronary arteries, a so-called mesenchymal cell, originating from the proepicardium throughout an epithelial-to-mesenchymal transition, is the progenitor of both VSMCs and fibroblasts.²⁵ Whether the embryological origin of adventitial fibroblasts in other vessels exhibits a similar pattern is unclear. An extension of this model would imply that all VSMC progenitors, namely the local mesenchyme and the distal mesenchyme from the proepicardium and the cardiac neural crest cells, are able to differentiate into fibroblasts. It is likely that these fibroblasts, reminiscent of their embryological origin, not only contribute to the formation of the tunica adventitia but also move to the tunica media, thus accounting for the NM-VSMCs observed in this layer and described above (Figure 1B). This property, however, is not common to all fibroblasts because in some species, and in some vessels, NM-VSMCs are never observed in the tunica media in normal conditions. Thus, these findings and the evidence that adventitial fibroblasts are a heterogeneous cell population (see below) suggest two embryological origins of these cells; one origin is a distinct progenitor and the other is the same progenitor that generates VSMCs (Figure 1A).

The synthesis, deposition, and organization of the fibroelastic scaffolding of the vascular wall probably drive segregation of fibroblasts in the definitive adventitia.²⁶ Elastogenesis consists of the elaboration, assembly, and maturation of the provisional matrix found since the early stages of vascular development. Although elastic fibers appear in the rat aortic wall after midgestation time, they accumulate only around the early fetal to postnatal stages.^{27,28} Completion of elastogenesis is coincident with a dramatic decrease in the VSMC replication rate in the media and VSMC differentiation.^{2,3} There is no apparent correlation between elastogenesis and cell proliferation in the adventitia.^{2,29} ECM organization, ending up with the separation between the multilayered tunica media and the tunica adventitia, should also be considered in the context of boundary formation.¹⁷ Deposition of elastin and collagens seems to be particularly important in this respect. Mice null for the elastin gene hamper the stabilization of the arterial wall as demonstrated by an altered intimal-medial boundary and proliferation of subendothelial VSMCs in these mice.³⁰ In addition, the ECM could have a more general role in the establishment of tissue boundaries through two functions. The first is to store and mobilize growth factors and other signaling molecules that otherwise continue to induce VSMC proliferation and, hence, medial layer hypertrophy. The second is to serve as binding sites and targets for adhesion molecules (N-cadherin) and integrins ($\alpha_v\beta_3$)¹⁴ that mediate cell attachment to collagen and separate the forming wall from the surrounding mesenchyme. Such a role for the ECM has been demonstrated in other organs. For example, tenascin-C and syndecan-3 are involved in the establishment of tissue boundaries between the perichondrium and the periosteum.³¹

Specific cell recruitment in the intima, media, and adventitia and deposition of the ECM are coordinated by a wide

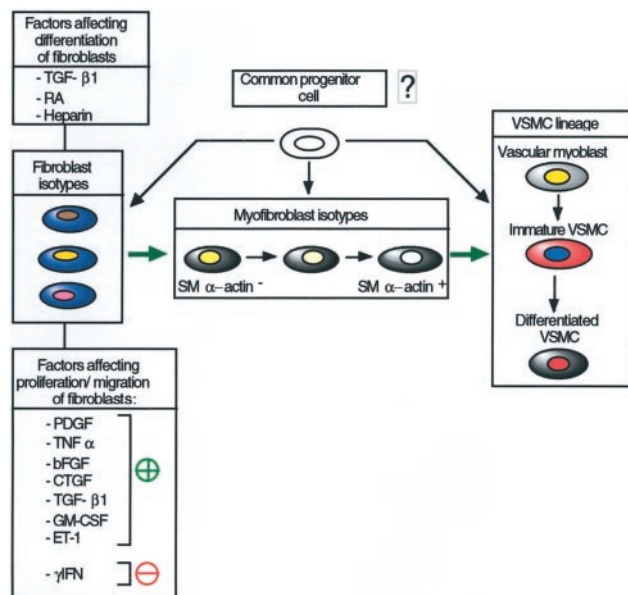


Figure 2. Diagram showing some possible or hypothesized cell-conversion pathways involving fibroblasts, myofibroblasts, and VSMCs. Some factors capable of influencing the proliferation, migratory, and differentiation pattern of fibroblasts are also shown. The number of fibroblast and myofibroblast isotypes is arbitrary but indicative of the great structural-functional heterogeneity existing among these cells. RA indicates retinoic acid; TNF α , tumor necrosis factor- α ; CTGF, connective tissue growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; and ET-1, endothelin-1.

variety of stimulators and inhibitors. If VEGF is mainly implicated in the assembly of ECs, PDGF-BB and transforming growth factor- β (TGF- β) family members are essential for the subsequent steps. PDGF-BB acts as chemoattractant for VSMCs and stimulates cell migration along the preexisting vessels.^{14–16} Members of the TGF- β signaling pathway (TGF- β_1 , TGF- β receptor 2, endoglin, and Smad5) work in unison to induce vessel maturation and stabilization by stimulating VSMC differentiation, ECM deposition, and concomitant inhibition of EC proliferation and migration.^{15,16}

Synopsis of Structural-Functional Features of Fibroblasts and Myofibroblasts

Before discussing the role of adventitial fibroblasts and myofibroblasts in neointima formation and vascular remodeling, it is useful to briefly review some biological aspects of these cells with respect to VSMCs (see Reference 32 for more details about this topic).

Fibroblasts make up the predominant long-lived stromal cell type the main feature of which is the production of the ECM components. It is a relatively recent notion that fibroblasts are a heterogeneous cell population with regard to both structure and function.^{31,32} *In vitro* they can variably show differences in SM α -actin content, morphology, and organization of adhesive structures.^{32,33} Activation of tissue fibroblasts induces the generation of a contractile force that correlates with SM α -actin expression and development of a cytoplasmically enriched microfilamentous system, fibronexus junctions, and other features shared by SM cells.^{33,34}

TABLE 2. Neointima Formation and Types of Vascular Injuries

Intraluminal Manipulation
Vessel overdistension ³⁹
Endothelial denudation ⁴⁰ (with medial injury)
Photochemical-induced thrombosis ⁴¹
Air-induced endothelial cell desiccation ⁴²
Implantation of metal coils ⁴³
Immunological injury ⁴⁴
Hypercholesterolemia ⁴⁵
Graft of prosthetic material ⁴⁶
Metabolic disorders ⁴⁷ (homocysteine-induced atherosclerosis)
Perivascular Manipulation
External electric stimulation ⁴⁸
External compression ⁴⁹
Stripping of the adventitia ⁵⁰
Positioning of a rigid polyethylene cuff, hollow silastic, or open collar around the artery ⁵¹
Coagulative necrosis of adventitia induced by argon laser energy ⁵²
Occlusion of vasa vasorum by flush ligation ⁵³
Endotoxin-treated thread ⁵⁴
Cold or hot injuries ⁵⁵

This change dismantles part of the morphofunctional apparatus of fibroblasts leading to the formation of hybrid NM-SM cells named myofibroblasts (Table 1). Numerous cytokines and growth factors can influence the proliferation level of fibroblasts as well as their transition to myofibroblasts (Figure 2, see References 35 and 36 for more details). It has been postulated that a cell continuum may exist from fibroblasts to myofibroblasts to immature VSMCs (Figure 2), as suggested by the peculiar sequential cell phenotypes activated in some models of tissue injury or during development.^{32–36} In addition, it has been suggested that fibroblasts, myofibroblasts, and VSMCs derive from a common stem (-like) cell.³⁶ At this time there is no experimental confirmation of this hypothesis, and it is not clear what relationship it may have to the putative mural progenitor cells discussed above.

There are numerous cytokines and growth factors that can influence fibroblast and myofibroblast proliferation as well as their phenotypic transitions acting directly (eg, TGF- β_1) or indirectly (eg, granulocyte-macrophage colony-stimulating factor; see Figure 2 and References 35 and 36 for major details). Activation of some SM differentiation markers in fibroblasts (ie, SM α -actin and stimulation of collagen type I synthesis) is positively regulated by TGF- β_1 ^{35,36} in concert with the ED-A portion of fibronectin.³⁷ Interestingly, TGF- β_1 also modulates the cellular retinol binding protein-1 (CRBP-1), a retinoic acid-responsive gene the activation of which in fibroblasts induces SM α -actin expression and conversion to myofibroblasts.³⁸

Role of Adventitia in the Response to Injuries

The involvement of adventitial fibroblasts in various aspects of response to injury and vascular remodeling will be discussed in the context of two conditions, direct endothelial and adventitial injury and autologous venous-to-artery graft and TA.

morphogenesis. Proliferating adventitial cells synthesize the PDGF-A chain and the PDGF- β receptor as part of the response in balloon-injured porcine coronary arteries.⁵⁷ Application of antisense oligonucleotides to PDGF- β receptor mRNA suppresses intimal thickening.⁷³

TGF- β_1 plays a central role in conferring differentiation, migratory, synthetic, and apoptotic features to adventitial fibroblasts. About 50% of activated fibroblasts express TGF- β_1 transcripts⁷⁴ 2 days after a severe balloon injury, concomitantly with myofibroblast appearance. Complete inhibition of SM α -actin expression, reduction in neointima formation, and reduced adventitial fibrosis and collagen deposition can be attained by injecting the recombinant soluble type II TGF- β receptor.⁷⁵ TGF- β_1 signaling in fibroblasts requires the presence of TGF- β receptor in combination with endoglin. Endoglin mRNA content is also markedly increased and displays a temporal pattern of expression coordinated with fibroblast proliferation, appearance of SM α -actin, and ECM synthesis.⁷⁶ TGF- β_1 is also able to regulate PDGF-A synthesis and the level of apoptosis in activated adventitial cells.^{77,78}

Changes in the integrin pattern, accompanied by a cytoskeletal rearrangement and activation of MMPs and other proteases, play a critical role in the translocation of activated adventitial fibroblasts to the medial layer. A correlation between β_3 integrin content and enhanced migratory ability of adventitial fibroblasts in vitro has been suggested.⁷⁹ Indeed, upregulation of integrins $\alpha_v\beta_3$ (and $\alpha_5\beta_1$)^{80,81} and increased synthesis of collagen I,⁶¹ fibronectin,⁸² and osteopontin⁸⁰ occur in injured adventitia.⁸¹ Polymerized or fibrillar type I collagen⁸³ and the glycosaminoglycan hyaluronan⁸⁴ may have important regulatory effects on integrin-mediated adhesion and directional migration of adventitial cells. Plasminogen activator inhibitor-1⁸⁵ and tissue factor⁸⁶ are markedly enhanced in injured adventitia, but it is not known whether their action may regulate the migratory ability of adventitial cells.

The role of estrogens in the control of proliferation and migration of adventitial fibroblasts remains quite ambiguous. Despite a lack of estrogen receptors, estrogens decrease the proliferation level of adventitial fibroblasts.⁸⁷ Control on adventitial fibroblast activation may be potentially achieved via soluble factors released from estrogen-sensitive medial VSMCs.⁸⁸ Li et al⁸⁹ suggested that inhibition of osteopontin, the expression of which is markedly increased after wall injury, by estrogen-sensitive VSMCs can affect the $\alpha_v\beta_3$ -integrin-mediated osteopontin directional movement of these cells. In these studies, however, the phenotypic characterization of activated fibroblasts and the existence of estrogen receptors in myofibroblasts have been not considered.

There are almost no data on the potential effect of angiotensin II on adventitial fibroblast activation, although it is well established that angiotensinogen is present in the adventitia and that the angiotensin-converting enzyme is induced in the injured adventitia. Indeed, local application of angiotensin II to intact carotid artery from normotensive rats induces adventitia thickening as a result of increased DNA synthesis, neovascularization, collagen deposition, and myofibroblast appearance.⁹⁰ An in vitro study suggests that angiotensin II causes a proliferation response in rat aortic

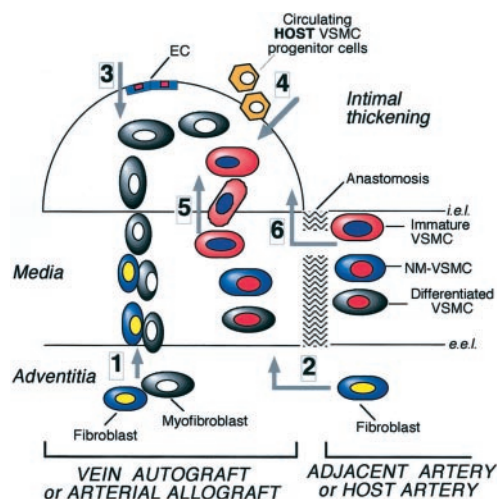


Figure 4. Diagram showing possible origin of intimal SM-like cells in vein-to-artery allograft and TA, beginning with venous/donor adventitial fibroblasts and myofibroblasts (1); adjacent/host adventitial fibroblasts (2); venous/donor (and host?) ECs (3); circulating host VSMC progenitors (4); and donor medial VSMCs, possibly via activation of resident immature VSMCs, NM-VSMCs, or differentiated VSMCs (5). i.e.l. indicates internal elastic lamina; e.e.l., external elastic lamina.

adventitial fibroblasts via binding to the angiotensin-1 receptor, which is potentiated by PDGF-BB and bFGF.⁹¹ It will be important to confirm in vivo such a role for angiotensin II in injured vessels and to test whether such an activation pattern may represent an autocrine/paracrine model for collagen turnover in injured adventitia. Reduction of adventitial cell proliferation and neointima formation can also be attained by treating animals with n^ω-3 polyunsaturated fatty acids before injury, the mechanism of which is unknown.⁹²

Adventitia Response in Autologous Vein-to-Artery Graft and TA

Many cellular responses evoked in arterial injuries take place in autologous vein-to-artery graft as well.⁹³ Both processes are characterized by an increased cell proliferation in adventitia, apoptosis and necrosis of medial VSMCs, adventitial thickening, fibroblast-to-myofibroblast conversion, directional migration of activated adventitial cells to the neointima, and vascular remodeling.^{11,12,93-98} In the transplant there is a transient acute inflammation, platelet adhesion, and release of vasoactive substances, cytokines, and growth factors.⁹⁵

In an elegant work, Shi et al⁹⁴ demonstrated that, after porcine autologous vein-to-artery grafting, SM α -actin⁺, BrdU⁺ pulse-labeled adventitial fibroblasts from adjacent tissue translocate from the interposed vein segment and, with time, migrate to the subendothelial space where numerous SM α -actin⁺, BrdU⁺-labeled myofibroblasts accumulate. Using this approach, however, the possible contribution of medial differentiated VSMCs and NM-VSMCs (Figure 4) could not be ascertained. These two VSMC subsets show a different proliferation and apoptotic rate in vein grafts.⁹⁸ Thus, the relative contribution of transplanted and adjacent adventitial fibroblasts/medial cellular subsets in the formation of intimal thickening remains an open question.

We have poor knowledge of the factors involved in growth and phenotypic cell conversion of adventitial fibroblasts in veins. Organ cell cultures confirmed the ability of the cells to migrate from the adventitia to the neointima via cut edges⁹⁹ or through the media.¹⁰⁰ The latter study revealed that expression of the 16-kDa subunit of vascular-type [H⁺]ATPase (member of a highly conserved family of polypeptides implicated in various transport functions) seems to be a prerequisite for fibroblast growth and subsequent differentiation into myofibroblasts. Unfortunately, the mechanism by which this vascular-type [H⁺]ATPase can act is not known.¹⁰⁰

There is no specific information about the potential role of growth factors and cytokines in promoting activation of adventitial fibroblasts in vein grafts. Intimal thickening seems to be correlated with the production of PDGF, bFGF, interleukin-1, and tumor necrosis factor α , whereas regression is dependent on TGF- β_1 production.¹⁰¹ Local hemodynamic changes (low shear stress) have no effect on neointimal cell proliferation and differentiation. In particular, the phenotypic profile of cells accumulated in the neointima, ie, immature VSMCs and fibroblasts, does not vary in comparison with standard stress conditions.¹⁰² Transient venous tissue ischemia, induced by the damage to the adventitial vasa vasorum and nerves when isolated from the original vein, could also modulate the production of activating factors from VSMCs, ECs, and adventitial fibroblasts. Unfortunately, there are no data to support this contention. Similarly, it is unknown whether the reactive oxygen species (ROS) produced in the adventitia (see below) by reperfusion injury have the same effect once the vein is grafted on the arterial position.

Adventitial fibroblasts may also play a role in TA, which is an important complication of organ allotransplants. This chronic vasculopathy is characterized by a diffuse nonfocal narrowing of the lumen due to neointima formation or expansion of a preexisting intimal layer (where present), fibroblast proliferation, deposition of ECM, and wall infiltration of inflammatory and immune cells.¹⁰³ The pathophysiology of this disease is based on both immunologic and nonimmunologic mechanisms. Backing up the former, immunosuppressive therapy in rat reduces the risk of graft failure due to acute rejection,¹⁰⁴ and an acquired immune response in mice (via CD4⁺ helper T lymphocytes, humoral antibodies, and macrophages) is essential for neointima formation.¹⁰⁵ In favor of the latter, application of the current immunosuppressive therapy in humans is not necessarily mirrored by the reduction in development of TA.¹⁰⁶

As for the autologous vein-to-artery graft, in TA there may be multiple potential, not necessarily exclusive, exogenous cell sources, apart from the resident species-specific intimal cells, for intimal thickening (Figure 4). There is a general consensus regarding early adventitial inflammatory cell proliferation and alteration of endothelium with thrombus formation (\approx 1 to 2 weeks). Less agreement exists regarding the chronological sequence and precise temporal window for other aspects of TA, ie, intimal thickening formation, medial VSMC proliferation and necrosis, and the inflammatory-immune cell infiltration to the inner wall layers.^{107–112} Medial VSMC necrosis,

which occurs relatively late, is not a prerequisite for intimal cell accumulation.¹⁰⁸ Instead, an important determinant of this event may be the phenotypic dedifferentiation of medial VSMCs,¹¹¹ with its inherent loss of contractile properties.¹⁰⁰ This is followed by medial VSMC replication¹⁰⁷ and appearance of immature VSMCs¹¹¹ or myofibroblasts,¹⁰⁹ along with inflammatory cells, in the intima. However, these data do not permit us to draw a firm conclusion about the phenotypic identification of SM-like cells accumulated in the intimal thickening. It is interesting to note that adventitial fibroblasts may act as “sentinel cells” by releasing soluble factors that regulate exogenous cell infiltration, ie, inflammatory and immune cells.¹¹³

A new impetus to the problem of SM-like cell derivation in intimal thickening comes from the Shimizu et al¹¹³ study. These authors, using mouse aortic allografts in allomismatched β -galactosidase transgenic recipient mice, demonstrate that most intimal immature VSMCs derive from a host circulating pool of bone marrow–derived cells.¹¹⁴ The contribution of medial VSMCs from host or donor mice is quite limited. However, the role of host/donor adventitial cells with respect to intimal thickening has not been investigated in this study. If proven, it may also shed some light on the possible regulatory role of donor/recipient adventitial fibroblasts on the marked and prolonged immune response that accompanies the development of this vasculopathy.

Conclusions

In the adult, cell movements and phenotypic changes of adventitial fibroblasts in response to vascular injury are very similar to what is observed during vascular morphogenesis (Figures 1 and 5). Both events go through a hybrid transient or permanent NM-SM cell. In the injured vessel, this unique cell is the myofibroblast that can stem from adventitial fibroblasts. In the developing vessel, this cell is the vascular myoblast that may derive from a common fibroblast-VSMC progenitor cell. Differentiated VSMCs, in turn, when injured dedifferentiate or phenotypically modulate, possibly in a clonal manner, giving rise to immature VSMCs. Alternatively or in addition, preexisting immature VSMCs or NM-VSMCs are activated (possibly in a clonal manner) and participate in the response to injury, the latter cell type also differentiating to hybrid NM-SM cells. We postulate that in the neointima or intimal thickening, the myofibroblasts and immature VSMCs represent the expression of an identical or very similar cell phenotype (Figure 5). Thus, this hybrid cell is in the heart of two convergent phenotypic pathways with opposite differentiation trends, the extremes of which are the adventitial fibroblast and the medial VSMCs. The marked differentiation plasticity emerging on demand in fibroblasts and VSMCs allows for a combined and more efficient response to injury. It is reasonable, therefore, to assume that this capacity to face injury has been maintained during evolution to deal with a continuous stress. If this hypothesis is correct, one may also ask whether the

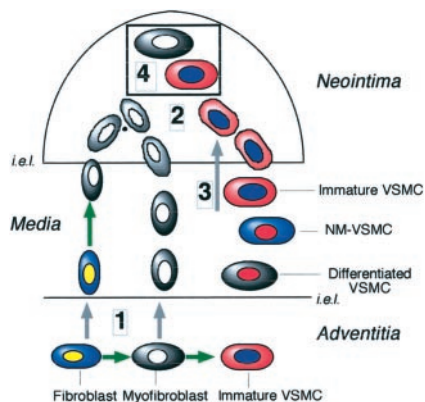


Figure 5. Diagram showing a model for cell contributions to neointima formation in response to injury. Gray arrows indicate direction of migrating cells; green arrows, cell phenotypic transitions. 1, Adventitial fibroblasts can be converted into myofibroblasts in adventitia or in media. 2, Fibroblasts/myofibroblasts are characterized by an overt migratory ability toward the sub-endothelial space. 3, Neointima can also be formed with the contribution of the VSMCs. VSMCs, either as repair-specific immature VSMCs existing before the injury or as differentiated VSMCs that undergo dedifferentiation/phenotypic modulation on demand, proliferate and migrate to the subendothelial space. NM-VSMCs may also participate in this event by differentiating to an SM-containing cell. 4, We postulate that two major phenotypic pathways (fibroblasts and NM-VSMCs/differentiated VSMCs) converge to the same or very similar cell phenotype. i.e.l. indicates internal elastic lamina; e.e.l., external elastic lamina.

adventitial fibroblast can be considered a renewing cell compartment¹⁵ composed of stem-like cells formed during development. In addition, given the structural and functional fibroblast heterogeneity,^{32,33} one may wonder whether the fibroblast activation observed in vivo in aorta, coronary artery, and carotid artery injury also occurs in other vessels or in different levels of the same vessel as it happens for VSMCs.¹⁻⁴

One may also pose the question of whether there is a unique tendency for activating adventitial fibroblasts in different experimental conditions. It is not known whether this putative cell compartment is called into action only in a “state of emergency,”¹¹⁶ when severe wall damage has been inflicted, or under other conditions as well, such as hemodynamic stress. The fact that a specific fibroblast subset in the pulmonary artery is deeply involved in adventitial cell activation in hypoxia-induced hypertension is in favor of this hypothesis.^{2,117} Hypoxia, generation of oxidative stress, and ROS formation can play important roles in fibroblast activation and recruitment because in these cells ROS is involved in the regulation of cell growth and death. In line with this hypothesis, Shi et al¹¹⁸ recently showed high levels of ROS produced by NAD(P)H oxidase in activated adventitial fibroblasts from balloon-injured porcine coronary arteries. Thus, it is conceivable that an adequate anti-ROS therapy is potentially able to counteract neointimal formation by acting both on VSMCs¹¹⁹ and fibroblasts.

Note Added in Proof

The recent article by De Leon et al¹²⁰ supports the view that species-specific differences exist in the ability of activated adventitial cells to migrate to the medial layer or subendothelial region.

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