Topological Determinants and Consequences of Adventitial Responses to Arterial Wall Injury

Jean-Baptiste Michel, Olivier Thaunat, Xavier Houard, Olivier Meilhac, Giuseppina Caligiuri, Antonino Nicoletti

Abstract—Arteries are composed of 3 concentric tissue layers which exhibit different structures and properties. Because arterial injury is generally initiated at the interface with circulating blood, most studies performed to unravel the mechanisms involved in injury-induced arterial responses have focused on the innermost layer (intima) rather than on the outermost adventitial layer. In the present review, we focus on the involvement of the adventitia in response to various types of arterial injury leading to vascular remodeling. Physiologically, soluble vascular mediators are centrifugally conveyed by mass transport toward the adventitia. Moreover, in pathological conditions, neomediators and antigens can be generated within the arterial wall, whose outward conveyance triggers different patterns of local adventitial response. Adventitial angiogenesis, immunoinflammation, and fibrosis sequentially interact and their net balance defines the participation of the adventitial response in arterial pathology. In the present review we discuss 4 pathological entities in which the adventitial response to arterial wall injury participates in arterial wall remodeling. Hence, the adventitial adaptive immune response predominates in chronic rejection. Inflammatory phagocytic cell recruitment and initiation of a shift from innate to adaptive immunity characterize the adventitial response to products of proteolysis in abdominal aortic aneurysm. Adventitial sprouting of neovessels, leading to intraplaque hemorrhages, predominates in atherothrombosis. Adventitial fibrosis characterizes the response to mechanical stress and is responsible for the constrictive remodeling of arterial segments and initiating interstitial fibrosis in perivascular tissues. These adventitial events, therefore, have an impact not only on the vessel wall biology but also on the surrounding tissue. (Arterioscler Thromb Vasc Biol. 2007;27:1259-1268.)

Key Words: mass transport ■ angiogenesis ■ lymphoid neogenesis ■ chronic rejection ■ abdominal aortic aneurysm ■ atherothrombosis

Remodeling of vascular tissues is defined as the structural consequences of vascular wall cellular and extracellular matrix dysfunction. Pathological remodeling of the arterial wall includes all the biological activities leading to reshaping of the arterial circumference, from wall shrinkage (stenosis) to enlargement (aneurysm). Whether stenosing or aneurysmal, remodeling is the pathological end point of the majority of arterial wall injuries. The purpose of this review is to recapitulate experimental evidence, to propose concepts and to describe pathological entities illustrating how the adventitia is alerted by and responds to luminal injury, thereby participating in arterial wall remodeling.

Topological Structure/Function Relationships in the Arterial Tissue

Although the arterial wall is composed of 3 independent layers (intima, media and adventitia), these 3 layers are differently structured and display a variety of physiological properties, leading to different responses to arterial wall injuries.

The intima is a monolayer of interconnected endothelial cells that adhere to a thin extracellular matrix. Physiologically, the most important role of the endothelium in arteries is to physically and functionally separate the circulating blood compartment from the tissue of the vascular wall. Arterial wall injury can result from mechanical stress, cell aggression or circulating molecular insulting agents. These injuries are operating at the luminal interface between circulating blood and the arterial wall. Therefore, the intimal response occurs early in the course of pathology induced arterial remodeling. This may explain why the majority of studies have focused on this intimal response. Regardless of its nature, the initial insult promotes either endothelial activation or de-endothelialization.

The media is the main structural component of the arterial wall, providing it with the ability to resist hemodynamic...
stress. Under physiological conditions, the medial layer is devoid of vasa vasorum.1–3 Some intramedial vasa vasorum are physiologically observed only in the outer part of the thoracic aorta in humans.3 The medial layer is delimited by the hydrophobic internal and external elastic laminae, and thus inaccessible to inflammatory cells as demonstrated in a mouse model of Herpes virus infection of large vessels,4 and in a rat model of chronic arterial graft rejection.5 The physiological inability of the media to be recolonized by mesenchymatous cells after injury-induced smooth-muscle cell (SMC) disappearance6–9 also supports this concept of medial inaccessibility to cells. Because cell adhesion and migration are highly dependent on the hydrophilicity of the microenvironment,10,11 the “medial privilege” is probably linked to the hydrophobicity of fibrillar elastin,12 and could explain the early intimal retention of cells and macromolecules, usually observed in response to luminal injuries.13,14 These properties led to the suggestion that the medial layer is poorly accessible to macrophages and lymphocytes. However, the media does remain accessible to soluble mediators such as interferon-γ or immunoglobulins5,15 and can thus be targeted by pathogenic processes.

The adventitia, the outermost part of the arterial tissue, is composed of a network of connective tissue, including collagen fibers, vasa vasorum,16 nerve endings, a few quiescent resident inflammatory cells and fibroblasts. Adventitial vasa vasorum constitute a complete vascular tree-like structure17,18 including arterioles, involved in the supply to the outer part of the arterial wall,19 capillaries and veins, involved in cell and molecule exchanges, including drainage of the wall soluble components.20 An absorbing adventitial lymphatic network consisting of large and sparsely distributed capillary structure is also present, suggesting that lymphatic drainage is involved in arterial wall homeostasis.21 The adventitia is a highly responsive tissue and regenerates very quickly in response to experimental stripping.22,23 Indeed, a normally functioning adventitia is crucial for the homeostasis of the entire vessel wall. Removal of adventitia22 or vasa vasorum occlusion by a collar23 induce intimal hyperplasia, involving both smooth-muscle cells and macrophages,24 which regresses with adventitia regrowth. Wall retention of plasma-derived macromolecules could be a consequence of a decrease in adventitial clearance ability.25 These experiments illustrate that events taking place in the adventitia have repercussions on biological processes in the intima and media. In solid organs, the adventitia of the vessels is in continuity with the interstitial tissue. Indeed, under physiological conditions, peptides, macromolecules or particles, conveyed from the blood to the adventitia through the arterial wall by mass transport, are taken up by adventitial vasa vasorum or lymphatics and driven back into the circulation or cleared in situ by phagocytic cells.

From Intima Toward Adventitia
Any initial luminal insult and the early intimal responses which ensue ultimately lead to important repercussions on the physiology of the media and of the adventitia because macro- and microparticles, soluble agents and mediators are convected through these layers toward the adventitia as the result of hydraulic conductance through the arterial wall. Therefore, although the initial insult is intimal, the adventitia becomes the ultimate site in which arterial responses to injury are elaborated (Figure 1).

Centrifugal Mass Transport in the Arterial Wall
Wash-in of soluble mediators through the arterial wall is driven by physiological transport forces, principally solvent-driven flow (convection) and random molecular agitation.
Neomediators in Arterial Wall Injury

Arterial wall injury often results in an increased hydraulic conductance and therefore in an increased transfer of mediators from the lumen to the adventitia\(^45\) attributable to an increase in permeability,\(^34\) or to a greater genesis of diffusible molecules. Arterial injury can generate new tissue-derived mediators related to SMC apoptosis,\(^8\) or resulting from the action of locally released reactive oxygen species and the consequent post-translational modifications of proteins, such as oxidation of plasma-derived (lipo)proteins. Finally, new tissue-derived molecules can originate from proteolytic degradation of the extracellular matrix. For example, solubilized elastin-derived peptides are chemotactic for inflammatory cells\(^46–48\) and capable of inducing neoangiogenesis.\(^49\) Similarly, fibrinogen-derived peptides released on proteolysis can increase permeability\(^50\) and are chemotactic.\(^51,52\) Oxidative modification of low-density lipoprotein or phospholipids\(^53\) can lead to the formation of auto-antigens\(^54\) and the stimulation of angiogenesis.\(^55\)

Therefore, the products of tissue cytolysis, proteolysis, or oxidation could all generate new antigens or mediators of the arterial response to injury. We propose that these tissue-borne neomediators, centrifugally convected by mass transport, are responsible for localization within the adventitia of the main arterial responses, including angiogenesis, inflammatory cell-dependent phagocytosis, the shift from innate to adaptive immunity, and fibrosis.

In order to provide in vivo evidence of this concept in arterial pathology, we have chosen 4 disease entities in which the adventitial responses are driven by outwardly conveyed information, generated by luminal injury (Table).

Adventitial Responses in Vascular Pathologies

Adventitial Lymphoid Neogenesis in Chronic Rejection

The most common histopathologic feature in chronic rejection is graft arteriosclerosis.\(^56\) Animal models based on aortic transplantation between histoincompatible murine strains have been developed to investigate arterial wall changes in chronic rejection. The main characteristics of both experimental allograft arteriosclerosis and of rejected human grafts can be summarized by: (1) diffuse narrowing of the arterial lumen, (2) delayed disappearance of SMCs from the media, and (3) persistent accumulation of mononuclear leukocytes in the adventitia.\(^5,57,58\) The early circulating cellular effectors, rapidly recruited beneath the endothelium, induce the early luminal destruction leading to the centrifugal mass transport of alloantigens toward the adventitia, but fail to reach the allogenic SMCs in the media because they are protected by the internal elastic lamina. Several lines of evidence point to the adventitia as a site of local adaptation of the immune response during chronic vascular rejection.\(^59\) At an early stage, the adventitial infiltrate is mainly composed of macrophages and cytotoxic lymphocytes. The ingress of leukocytes into the adventitia is favored by the change in the phenotype of adventitial endothelial cells of the vasa vasorum that acquire a high endothelial venule–like phenotype.\(^59\) High endothelial venule are specialized endothelial cells physiologically located in the secondary lymphoid organs, where they support an intense recruitment of naive lymphocytes. During the initial phase of the adventitial infiltration, the various leukocyte populations are not noticeably spatially coordinated.\(^59\) These cellular effectors are unable to cross the external elastic laminae of the media in absence of angiogenesis. The inability of inflammatory cells to eradicate the alloantigens, which are centrifugally convected, creates the optimal conditions for lymphoid neogenesis to take place within the adventitia.\(^60\) Lymphoid neogenesis is a term coined by Kratz et al\(^61\) to describe the progressive organization of chronic inflammatory infiltrates in nonspecialized tissues into structures that morphologically resemble germinal centers of the secondary lymphoid organs. The adventitial tissue supports the development of these structures which are characterized by the presence of B lymphocyte nodular infiltrates that are ectopic germinal centers.\(^62–64\)
similarities with secondary lymphoid organs, ectopic lymphoid tissues are functional because they support local clonal expansions, somatic hypermutations, and antibody production. Although not completely understood, the molecular mechanisms underlying organization of chronic inflammatory lesions into ectopic lymphoid tissue appear to recapitulate some of those involved in lymphoid organogenesis during development. These locally produced alloantibodies diffuse into the general circulation, cross the internal elastic lamina and bind to medial allogenic SMCs. This binding leads to (1) a rapid upregulation of the transcription of growth factor genes by SMCs, followed by (2) the induction of apoptosis. As the medial alloimmune targets progressively disappear, the source of alloantigens diminishes and the adventitial alloimmune response progressively turns off. The subsequent fibrotic scarring process in the adventitia results in a constrictive remodeling process that synergizes with the neointimal proliferation to cause reduction in lumen diameter of the rejected arteries.

Besides chronic rejection, it is noteworthy that adventitial lymphoid neogenesis has also been evidenced in inflammatory arteritis such as Kawasaki disease, Takayasu, and giant cell arteritis.

Adventitial Inflammation and Fibrosis in Abdominal Aortic Aneurysm

Aneurysms of the abdominal aorta are the consequence of cell loss and proteolytic degradation of the insoluble extracellular matrix within the medial layer of the aortic wall. In response to this proteolytic injury, outward localization of inflammation, edema, and lymphoid neogenesis are initiated in the adventitia. In this context neomediators, generated by proteolytic injury of the media and convected into the adventitia, could play an important role. Inflammatory cell retention and lymphoid neogenesis are linked to capillary development in the adventitia in abdominal aortic aneurysm (AAA). Neoangiogenesis in the outer part of AAA was described 10 years ago. The authors showed strong spatial correlations between neocapillaries, degradation of elastin and the extent of the inflammatory infiltrate in the outer aortic wall, which predominated in AAA as compared with aortic occlusive atherosclerosis. However, in contrast to the situation in intimal plaques or occlusive thrombus, neovessels do not colonize the media and the luminal thrombus in AAA, probably because of a local excess of proteolytic activities.

The main early function of inflammatory cells in the adventitia of AAA is probably phagocytosis. This function is illustrated in Figure 2c, in which Prussian blue-stained hemosiderin is observed in macrophages of the inner adventitia, providing evidence of the convection of red blood cell–degradation products from the luminal thrombus to the adventitia. Phagocytosis of degraded cells and molecular products influence the immune response. The observation of chronic periaortic infiltrates, consisting mainly of lymphocytes, monocytes, plasma cells and sparse eosinophils, usually on a background of abundant fibrous tissue and fibroblasts, has prompted the use of the terms “inflammatory AAA” and “retropertitoneal fibrosis”, and the concept of an adventitial shift from innate to adaptive immunity in AAA. Koch et al observed that the adventitia in AAA was enriched in lymphoid aggregates. Lymphoid follicles, containing dendritic cells and activated endothelium, were thereafter described (Figure 2d). The similarity of adventitial lymphoid structures with germinal centers of secondary lymphoid organs has led to the proposal that lymphoid neogenesis takes place in the adventitia of AAA. In the light of these observations, an attempt was made to characterize the adventitial immune response in AAA. Lymphocyte populations, Th1/Th2 balance, presence of B cells and clonality were explored. Thus, the definitive demonstration that adventitial lymphoid neogenesis takes place in the adventitia of AAA is underway. Nevertheless the antigens
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Adventitial Angiogenesis in Atherosclerosis

Physiologically, the major part of the medial layer is devoid of any vasa vasorum. Oxygen diffuses from the lumen to the inner part of the wall, and vasa vasorum, present in the adventitia, is in charge of the oxygenation of the outer part of the arterial wall.

In 1981, Heistad and coworkers described an increased perfusion in the outer layer of the aorta of hypercholesterolemic atherosclerotic monkeys. In the early stage of atherosclerosis, hypercholesterolemia promotes the development of advential coronary vasa vasorum in a porcine model, more than does hypertension. This phenomenon seems restricted to coronary artery, particularly to its proximal epicardial segment in relation to vasa vasorum density. Therefore, adventitial neoangiogenesis appears to be linked to the evolution of atherosclerosis from early stages toward complicated lesions.

As early as 1938, in parallel with the observations of intraplaque hemorrhage in vulnerable plaques, Paterson reported the development of neocapillaries in complicated plaques and their involvement in subintimal hemorrhage. Such observations allowed Barger et al to propose a role for neoangiogenesis in the development of the pathological consequences of atherosclerosis. In their remarkable study, using postmortem microangiography and corresponding histological examination of coronary arteries, Kumamoto et al demonstrated that neovascularization of the atherosclerotic plaque originated mainly from the adventitia and rarely from the lumen. In humans, these neocapillaries develop mainly in the shoulder of the complicated plaque, at the interface between the core, the cap and the media. These neocapillaries would allow diffusion of plasma-borne molecules and leukocyte diapedesis. Indeed, the density of intimal neocapillaries correlated with the extent of core formation, hemosiderin deposits, hemorrhages and inflammatory infiltrates, suggesting that centripetal angiogenesis is linked to atherosclerosis evolution. Similar data of neovascularization have been reported in human carotid plaques and aorta, correlating in all cases with plaque evolution.

These observations have been recently extended to atherosclerosis-prone knock-out mouse models, in which a correlation between adventitial vasa vasorum neovascularization and plaque progression in the aorta was reported. Interestingly, Moulton and coworkers demonstrated that inhibition of plaque neovascularization reduced plaque infiltrates and progression in apolipoprotein E-deficient mice, therefore indicating that neoangiogenesis within the vessel wall is a critical determinant of plaque formation and subsequent evolution. It has been suggested that plasminogen activators and plasmin formation play a role in the neoangiogenesis process as well as proinflammatory cytokines such as interleukin.

In contrast to capillary neovascularization, lymphangiogenesis is rare in atherosclerotic plaques. It is therefore tempting to speculate that insufficient lymphatic drainage of the plaque participates in the retention of mediators in the adventitia and promotes lymphoid neogenesis. These results, together with the data on hypercholesterolemia-induced adventitial angiogenesis, provide evidence that (1) the adventitial angiogenic response is an earlier event than usually supposed in atherosclerosis and is associated with all the stages of the plaque evolution, (2) plaques may generate mediators able to induce the formation of neocapillaries, and (3) these mediators are centrifugally convected from plaques toward the adventitia.

Whereas monocytic cell migration from blood to the intima is thought to be mainly aimed at phagocytosis and initiation of the early atheromatous process, inflammatory infiltrates are present in the shoulder region of the lesion core and in the adventitia adjacent to complicated plaques. Adventitial mononuclear cell infiltration associated with atheromatous plaques was reported by Gerlis in 1956 in coronary arteries and by Schwartz and Mitchell in 1962. A similar adventitial response was further documented in the aorta and was termed “chronic periaortitis” by Parums and Ramshaw and linked to retroperitoneal fibrosis. Although less studied than in AAA, ectopic germinal centers have also been described in the adventitia surrounding occlusive atherosclerotic plaques in aortic disease. Moreover, as in AAA, adventitial follicular infiltrates predominated in opposition to the medial thinning in regions where elastin fibers were degraded. Similar data have been reported by Higuchi et al in atheroma, in which adventitial infiltrate intensity correlated with the stage of atherosclerotic plaques (Stary classification) and the presence of mast cells. Therefore, as in AAA, the preferential adventitial localization of the immunoinflammatory response suggests the existence of centrifugal stimuli, probably linked in part to extracellular matrix degradation, and centripetal responses, including initiation of adaptive immunity in stenosing atheroma. The possible chronology of the disease process is provided by experiments...
in mouse models of occlusive atherosclerotic disease. Although adventitial nonclustered T cells predominate in young apolipoprotein E–deficient mice, clusters containing T and B cells as well as lymphoid-like structures are observed in older mice, preferentially in the abdominal aorta.63,114

Responses to Mechanical Injuries
Adventitial fibroblasts play a critical role in the adventitial response to injury. They can differentiate into myofibroblasts, migrate, proliferate and secrete procollagen-1, which forms a network of insoluble collagen in the extracellular space, leading to perivascular fibrosis. The main cytokine capable of activating fibroblasts in actin-positive myofibroblasts,115 and to induce collagen synthesis and secretion116 is transforming growth factor β-1. Transforming growth factor β-1 synthesized by polarized macrophages and immune cells is probably the main molecular link between inflammation, involved in the detersion of the injured tissue, and the perivascular fibrotic healing process.117 The development of perivascular fibrosis has been mainly studied in response to mechanical injuries, namely hypertension and balloon injury. In these situations, collagen turnover118 and collagen fiber neochitecture119 are involved in constrictive remodeling of the arterial wall. Studies suggest that fibrosis is also a feature present in the arterial response to other injuries such as graft arteriosclerosis,67 and AAA-dependent retroperitoneal fibrosis.76

High blood pressure induces medial SMC mechanical stretch, hypertrophy and changes in expression pattern. Arterial walls exposed to hypertension, overexpress adhesion molecules such as intercellular adhesion molecule-1120 and proinflammatory cytokines such as monocyte chemoattractant protein-1.121 This hypertension-induced proinflammatory phenotype of the media is mediated by oxidative species and nuclear factor κB activation within the stressed SMCs.122,123 These mediators, outwardly conveyed, lead to the perivascular retention of inflammatory cells,124 transforming growth factor β-1 overexpression (by inflammatory cells), perivascular activation of fibroblasts and fibrosis. This pathophysiology has been mainly studied in small arterioles of the coronary bed121 but also exists in the kidney. In the heart and in the kidney, arterial responses to hypertension initiate and contribute to interstitial reactive tissue fibrosis. Such a mechanism has been less investigated in adventitia of large vessels, but a similar fibrotic adventitial response has been reported in pulmonary hypertension.125

We know that adventitia also participates in the response to balloon injury (angioplasty in human).126 Indeed, at least a part of the reactive actin-positive cells migrating to the intima could be of adventitial origin.127 In response to balloon injury, the proliferation of myofibroblasts was reported to be greater in the adventitia behind the medial tear than in the media itself, leading to an increase in collagen turnover and matrix metalloproteinase expression.128 The adventitia was also the site where the platelet-derived growth factor and its receptors were the most highly expressed. As in hypertension, leukocytes infiltrated the adventitial layer in response to balloon injury.129 This infiltrate was composed of neutrophils and macrophages. In a pig coronary model of balloon injury, the adventitial inflammatory infiltrate was not confined to the immediate adventitia but was also found in the perivascular interstitial tissue, extending away from the arterial wall.129 Of note, as demonstrated in both animals and in humans, fibrosis-induced constrictive vascular remodeling, developed in the adventitia, is for a large part, responsible for the lumen loss associated with restenosis.130–132 Indeed, angioplasty studies in rabbits and pigs indicate that the size of the neointima does not completely explain the loss in luminal diameter measured morphometrically or by angiography,133 and that a shrinkage of external elastic lamina circumference, attributable to neoadvential formation, also participates in this reduction.134 This effect was prevented by anti-integrin antibodies.135 Data derived from intravascular ultrasound measurements in humans support these experimental observations and suggest that clinical restenosis is also associated with a constrictive remodeling occurring outside the injured vessel.130,135 It is noteworthy that constrictive remodeling was found to be associated with adventitial angiogenesis,136,137 which predominates in response to stenting. In a recent study, Cheema and coworkers demonstrated a correlation between the intrastent proliferation and the development of adventitial neovascularization.138

Conclusion
Most injuries to the vascular wall are driven by insulting agents acting from inside the lumen. Because injury-
generated mediators are centrifugally transported, an important part of the vascular wall "response to injury" takes place in the adventitia. Although the insulting stimuli may be diverse in nature, generating various patterns of neomediators, the adventitial responses are strikingly constant, associating angiogenesis, inflammation, and fibrosis (Figure 3). The pattern (Table) of the adventitial reaction depends on the nature of the insult, the types of neomediators generated, and also, perhaps predominantly, on the duration of the stimulus. According to the predominance of one or other of these processes, each arterial pathological entity is characterized by a specific remodeling pattern. Accumulating evidence suggests that the adventitial responses cannot be considered merely as markers of the ongoing pathological process. Indeed, we believe that the adventitial response influences, directly or indirectly, the biology of the entire arterial wall and of the surrounding tissue. Therefore, it is important to take into account this pathophysiological topology of adventitial responses, in the understanding of the nature of arterial wall injuries and how the arterial wall remodels.

**Sources of Funding**

These studies have been supported by Inserm, by the Fondation pour la Recherche Médicale and by the Leducq Foundation.

**Disclosures**

None.

**References**

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*Arterioscler Thromb Vasc Biol.* 2007;27:1259-1268; originally published online March 29, 2007;
doi: 10.1161/ATVBAHA.106.137851

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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