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Review

The role of the adventitia in vascular inflammation

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Abstract

Traditional concepts of vascular inflammation are considered "inside-out" responses centered on the monocyte adhesion and lipid oxidation hypotheses. These mechanisms likely operate in concert, holding the central tenet that the inflammatory response is initiated at the luminal surface. However, growing evidence supports a new paradigm of an "outside-in" hypothesis, in which vascular inflammation is initiated in the adventitia and progresses inward toward the intima. Hallmarks of the outside-in hypothesis include population of the adventitia with exogenous cell types, including monocytes, macrophages, and lymphocytes, the phenotypic switch of adventitial fibroblasts into migratory myofibroblasts, and increased vasa vasorum neovascularization. The resident and migrating cells deposit collagen and matrix components, respond to and upregulate inflammatory chemokines and/or antigens, and regulate the local redox state of the adventitia. B cells and T cells generate local humoral immune responses against local antigen presentation by foam cells and antigen presenting cells. These events result in increased local expression of cytokines and growth factors, evoking an inflammatory response that propagates inward toward the intima. Ultimately, it appears that the basic mechanisms of cellular activation and migration in vascular inflammation are highly conserved across a variety of cardiovascular disease states and that major inflammatory events begin in the adventitia.

Keywords: Adventitia; Vascular inflammation; Fibroblast; Myofibroblast; Lymphocytes

1. The adventitia in the inflammatory response to vascular injury

Vascular inflammation has traditionally been considered an "inside-out" response, centered on the monocyte adhesion to the intima of blood vessels [1] and the oxidative lipid hypotheses [2]. In the former, injured vascular cells on the intimal surface of the blood vessel express surface adhesion molecules and inflammatory mediators that participate in monocyte homing to the endothelium and eventual transmigration into the media. In the latter hypothesis, oxidized lipids in the circulation accumulate in macrophages on the intimal surface, injure the endothelium, and initiate an inflammatory process (Fig. 1). These mechanisms invoke a central role for vascular inflammation and indeed, it is likely that they operate in concert, holding the central tenet that inflammatory responses are initiated at the luminal surface. As a result, the luminal surface is where the majority of investigation into cardiovascular disease (CVD) pathogenesis has been focused.

However, growing evidence supports a new paradigm of an "outside-in" hypothesis, in which vascular inflammation is initiated in the adventitia and progresses inward toward the intima. The functional significance of the adventitia in vascular disease has been explored intermittently for many years. However, the hypothesis that the adventitia is a functional homeostatic regulator in CVD pathogenesis was only postulated about a decade ago in the setting of restenosis after percutaneous coronary angioplasty [3,4], and more recently in the setting of abdominal aortic aneurysm (AAA) development [5]. Indeed, studies show that in various presentations of CVD, the

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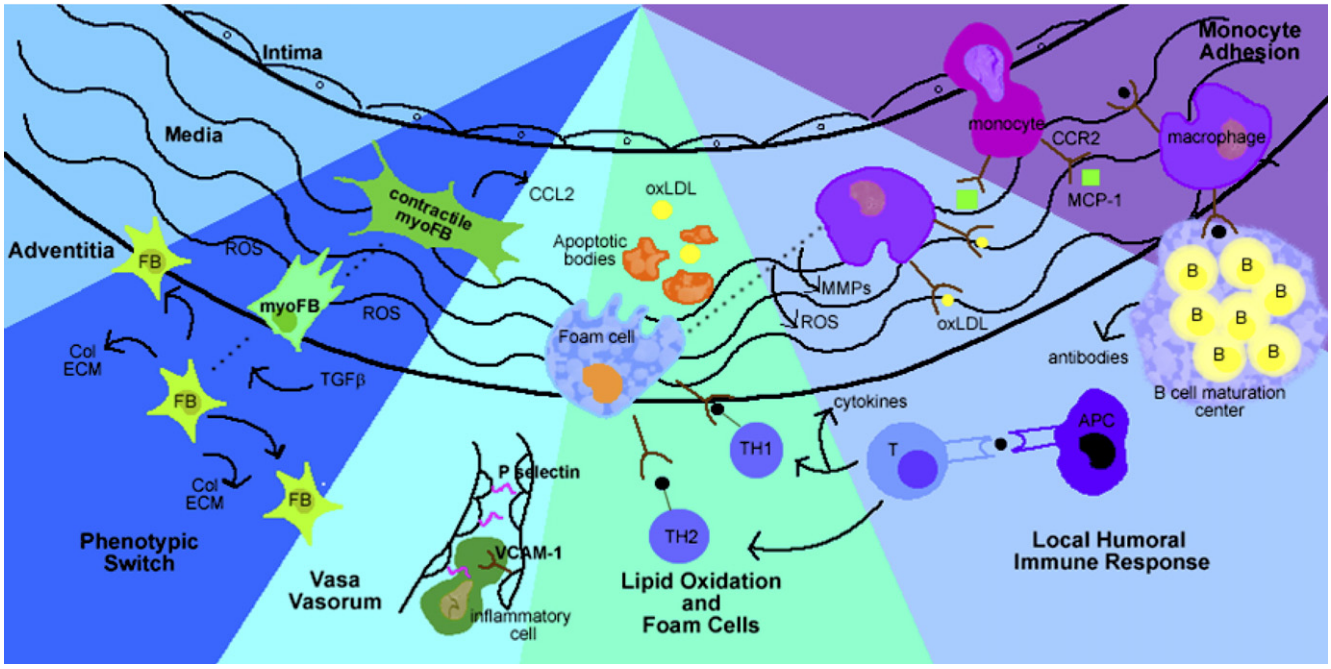

Fig. 1. The role of the adventitia in vascular inflammation in an arterial cross section. Moving from left to right, depicting the healthy adventitia, the phenotypic switch from fibroblast to myofibroblast, vasa vasorum neovascularization, lipid oxidation, generation of foam cells, B cell maturation and contribution to the local humoral immune response, and transmigration of monocytes into the arterial media. According to the outside-in hypothesis, inflammatory cues, perhaps in the form of a focal elastic laminae tear or chronic inflammation products, such as leukotrienes and chemokines, signal the adventitial fibroblast (FB) to undergo a phenotypic switch into a migratory myofibroblast (myoFB) in a transforming growth factor-β (TGF-b) dependent manner. The myoFB transforms into a contractile myoFB (cmFB). The cmFB generates additional cytokines, such as CCL2, eventually undergoing apoptosis by a redox mechanism. The remaining adventitial FB cells also secrete cytokines to recruit a massive influx of exogenous leukocytes. FBs also secrete excess collagen (Col) and extracellular matrix (ECM) to stabilize the artery and perhaps provide additional matrix on which to seed newly recruited cells. For increased cellular trafficking, the endothelium of the vasa vasorum demonstrates upregulated P-selectin and vascular cell adhesion molecule-1 (VCAM-1). Lymphoid organ-like clusters of lymphocytes, called B cell maturation centers, are where local humoral immune responses are generated. Antibodies against inflammatory antigens, presented by antigen presenting cells (APCs) are produced in these lymphoid clusters. APCs also interact with and activate T cells, which then transform into pro-inflammatory Th1 cells. The cytokines and antigens produced by activated lymphocytes serve as chemoattractants to promote monocyte transmigration through the endothelium and subsequent transformation into macrophages. Macrophages uptake oxidized LDL (oxLDL) and transform into foam cells and the apoptotic bodies resulting from foam cell death produce additional inflammatory antigens, release oxLDL, and modify the redox state of the local environment. At this point, outside-in and inside-out mechanisms collide in the vascular inflammatory cascade.

adventitia not only becomes populated with multiple exogenous cell types including monocytes, macrophages and T cells [6–8], but also shows increased replication and differentiation of fibroblasts and into myofibroblasts [9]. These events result in increased local expression of inflammatory cytokines and growth factors in a motif that is similar to that seen with inside-out mechanisms [10]. It is the objective of this review to explore the data supporting this non-traditional view of CVD-associated inflammation occurring in the adventitia and to consider the implications of this alternative pathogenic paradigm in our understanding of the basic mechanisms of vascular disease.

2. The cellular composition of the active adventitia

2.1. Fibroblasts and myofibroblasts

Many observations on the role of the adventitia in response to injury were first reported in the setting of restenosis after coronary angioplasty. Stent placement or balloon angioplasty injures the endothelium and produces a focal rupture in the internal elastic lamina. In response, vascular cells proliferate and migrate to the intimal region, secreting extracellular matrix (ECM) proteins and subsequently generating a neointima. Recent studies have shown that nearly half of the neointimal cells originate from proliferating adventitial cells [3], whereas previous studies hypothesized that most neointimal cells originated from the adjacent smooth muscle cells in the media [11]. Importantly, neointimal formation is accompanied by a significant increase in the size of the adventitia as well as a change in its cellular components, providing morphological evidence that the adventitia is an active participant in the wound healing response.

In the post-angioplasty setting, adventitial cells in regions near the medial tear demonstrate an increase in α-smooth muscle (SM) actin and desmin positive staining within 2 weeks. This is indicative of a phenotypic switch of fibroblasts into myofibroblasts (Fig. 1), a conversion considered the hallmark of adventitial response to injury in this model [4,12,13]. A myofibroblast is a modified fibroblast that has contractile properties as indicated by the presence of stress fibers and cytoskeletal proteins, which are

upregulated in the transformation from the non-proliferative fibroblast [14]. Myofibroblasts stain for α-SM actin, whereas fibroblasts do not [15]. Demonstration of myofibroblasts in the neointima of experimental angioplasty-induced intimal lesions was the first published evidence of the migratory capacity of the adventitial fibroblast. In addition, these studies provide the foundation for the hypothesis that the adventitia holds a fundamental role in intimal vascular lesion formation [4,12].

The activation and signaling mechanism of the myofibroblast has received much attention for the intriguing reason that it presents with the same phenotype and function regardless of tissue residence. Much of what is known about myofibroblast function was pioneered in the setting of wounding healing [16]. Powell et al. provided an elegant review of myofibroblast activity in intestinal cells [17,18]. Although specific myofibroblast activity in the vasculature has not yet been thoroughly detailed, there is most likely a strong correlation between the wound healing mechanisms occurring in gastrointestinal cells, and those occurring in the setting of CVD.

The lifespan of the myofibroblast appears to be controlled from the time of phenotypic switch until apoptotic death. Of particular interest to this discussion is growing evidence that fibroblast and myofibroblast regulation of collagen influences their role in the inflammatory response. In vitro studies have shown that ECM deposited by the myofibroblast has a distinct composition, implying the potential to uniquely control fibrolytic collagen deposition in the wounded vessel [18]. It appears that the myofibroblast may first deposit the basement membrane collagen type IV, which supports cell migration and attachment. The collagen is subsequently converted into mature and mechanically stable collagen type I. After myofibroblasts migrate and participate in wound healing and contraction, they undergo apoptosis which presumably regulates contractile remodeling by self-limiting excessive ECM deposition. Though not yet clearly defined, studies have shown that this apoptotic signal is dependent upon transforming grow factor-β (TGF-β), an established activator of fibroblast differentiation into myofibroblasts (Fig. 1) [19]. Somewhat paradoxically TGF-β also serves as a sustained chemoattractant for subsequent myofibroblast infiltration [20], illustrating the inherent theme of inflammatory balance wherein inflammatory mediators that enhance one process may serve to mitigate another.

The presence of non-migratory, but proliferating fibroblasts illustrates a second regulatory adventitial cell phenotype. Responding to either intimal or adventitial injury, these non-migratory fibroblasts impart an increased cellularity and cell density in the adventitia [21]. Fibroblasts themselves have been shown to be rich in caveolae content, suggesting the potential of the adventitial fibroblast to modulate protein trafficking mechanisms involved in vascular response to injury [22]. In the setting of injury, adventitial fibroblasts deposit procollagen alpha 1(I) [23] and presumably other ECM proteins into the adventitia (Fig. 1) [14,24]. As a homeostatic mechanism, these same cells also control excessive collagen deposition by upregulating collagen triple helix repeat containing 1, a novel regulatory protein that reduces collagen type I expression [24]. The concept that fibroblasts and myofibroblasts process and organize collagen was tested by inhibiting TGF-β in the setting of balloon angioplasty [25]. In addition to activating the fibroblast to myofibroblast conversion, TGF-β has also been shown to regulate ECM deposition by vascular cells [26]. The result of TGF-β inhibition was decreased constrictive remodeling associated with a reduction in post-angioplasty restenosis, preserved lumen, and dense collagen deposition in the adventitia. In addition, the collagen content in restenotic vessels was lower than in nonstenotic vessels. These data suggest that the non-migratory fibroblast influences the local adventitial collagen matrix, while the migratory myofibroblast organizes the collagen matrix along its migratory path toward the intima, where it becomes fully contractile, promoting restenosis and constrictive remodeling.

The phenotypic switch of fibroblast to myofibroblast is certainly not a solitary event in the adventitial response. Monocytic infiltration of the adventitia is likely preceded by migration of neutrophils and lymphocytes into the adventitia. Similarly, there are likely multiple locally released chemoattractants responsible for the migration of cells into the adventitia including platelet derived growth factor (PDGF) and monocyte chemoattractant protein-1 (MCP-1). Indeed it has been shown that after balloon injury myofibroblasts, along with other inflammatory cells, are found in granulation tissue. In the vasculature, adventitial granulation tissue is associated with altered ECM protein expression, promoting adventitial and peri-adventitial contraction and scarring [14]. This ultimately results in constrictive remodeling of the vessel and associated lumen loss.

The ability of the myofibroblast and non-migratory fibroblast to respond to injury in such an elegantly self-regulated manner is evidence that the adventitia plays a critical role in the vascular inflammatory cascade. It is clear that by directing the composition, function, and lifespan of its cellular composition, the adventitia has the potential to orchestrate pivotal matrix remodeling mechanisms in the response to injury. The fibroblast to myofibroblast conversion is such a fundamental mechanism in the adventitial role in vascular inflammation that it receives singular attention in *Adventitial Fibroblast Reactive Oxygen Species as Autacrine and Paracrine Mediators of Remodeling: Bellwether for Vascular Disease?* by Haurani and Pagano elsewhere in this issue.

2.2. Adventitial lymphocytes

Immune control of vascular pathology has been investigated since T cells were first observed in atherosclerotic lesions two decades ago [27]. As was the case with the monocyte adhesion hypothesis, many studies of lymphocyte involvement attempted to either remove what was considered

adventitial artifact or simply overlooked the adventitial contribution altogether. Recent efforts have provided numerous reports of the role of lymphocytes in both healthy and diseased tissue [4,28–33], and while abundant data on the adventitial fibroblast role has been obtained in the setting of balloon angioplasty, the majority of literature on lymphocytes in CVD has been in the setting of atherosclerosis. The mechanisms of T cell action in atherogenesis was recently thoroughly reviewed [34] and it has been hypothesized that circulating $CD4^+/CD31^+$ T cells may regulate local T cell action in the arterial wall [35]. Pro-atherogenic Th1 cells are shown to limit lesion size, but not lesion initiation [36]. In contrast Th2 cells are considered anti-atherogenic in that they have been shown to promote antibody-generating B cell production, as well mitigate cholesterol uptake.

While the presence of T cells within the adventitia of atherosclerotic lesions has been confirmed by multiple investigators[37,38], the presence of B cells is more enigmatic and is possibly related to the stage of lesion advancement [6,37,39,40]. The protective immunity conveyed by the B cell against atherosclerosis has, however, been corroborated by numerous investigators. In both apolipoprotein E (apoE) knockout mice and low density lipoprotein (LDL) receptor null mice, B cell depletion via splenectomy results in increased atherogenesis compared to unsplenectomized controls [41,42]. In the hypercholesterolemic apoE knockout model, post-splenectomy B cell transfer reduced both ultimate lesion size and $CD4^+$ T cell infiltration, yet did not yield any B cell populated lesions [42]. Interestingly, the studies that do report B cells within the atherosclerotic lesions show the cells localized in basal areas rich in vascular cell adhesion molecule (VCAM) expression, suggesting their role in activation of atherogenic pathogenesis in the injury response [38]. Taken together these findings suggest the existence of localized vascular B cells that work in concert with circulating B cells to both protect against atherogenesis and to reduce lesion formation limiting T cell activity, respectively [41].

What is most intriguing for this discussion is not the presence of T and B cells, but rather their location and organization. These lymphocytes have been found in the adventitia and media of wild type mice preceding any intimal lesion formation [31,33,39]. T/B cell clusters have been found in the setting of murine hyperlipidemia in the adventitia of non-atherosclerotic upper abdominal aorta, while complex lymphoid-like structures appear in the non-atherosclerotic abdominal aorta below the diaphragm [33]. This finding is particularly relevant to murine AAA which predominately form in the same subdiaphragmatic portion of the aorta. The spatial distribution of resident lymphatic cells in the adventitia of aortic regions vulnerable to disease may imply an adventitially localized feed-forward immune response that is spatially predictive of disease initiation.

In the setting of vascular injury, adventitial nodules with germinal centers composed primarily of B cells and associated macrophages and T cells have been shown near sites of advanced lesions in humans. Similar follicle-like structures are seen in murine lesions as well [33]. It is thought that these are antigen-driven B cell maturation centers (Fig. 1), where humoral immune responses are generated. Staining of microvessels near human plaques shows cell clusters with lymphoid organ-like pathology that are not found in non-atherosclerotic controls [40]. Such lymphoid clusters are typically associated with chronic inflammation states, and as such the observation of these structures in the adventitia of acutely inflamed arteries is unique. Cluster organization appears to depend upon the stage of plaque progression, but overall these adventitial nodular infiltrates are composed of well-organized B cells, T cells, and macrophages, within which the B cells mature and generate antibodies against inflammatory antigens. The specific antigens that trigger these responses are still undetermined, but stress proteins (heat shock proteins) [6], modified lipoproteins, and other surface antigens have been implicated.

Taken together, multiple studies link the origin of locally generated immune responses to the adventitia. Importantly, in both acute traumatic angioplastic injury and chronic vascular disease, coordination among resident fibroblasts, adventitial lymphocytes and inflammatory mediators orchestrates an inflammatory cascade that travels from the outside-in toward the intima to the site of injury (Fig. 1). Indeed the adventitial stimulus for post-angioplasty restenosis is acute trauma. In this setting, adventitially derived myofibroblasts may appear to be the predominant cell type regulating post-angioplasty constrictive remodeling. However, the role of the lymphocytes in post-angioplasty restenosis is currently undefined and could possibly play a comparable role to the myofibroblast in the inflammation response. In the same manner, it may be speculated in the setting of atherosclerosis that chronic stimuli, perhaps paracrine signaling from a dysfunctional endothelium, may provide the pro-inflammatory stimuli to activate adventitially residing fibroblasts and lymphocytes. At early atherosclerotic stages these activated cells can maintain an open lumen via outward remodeling. However, at later stages the myofibroblast is stimulated to migrate and promote neointimal formation and eventual lumen constriction. Each CVD pathogenesis has its own adventitial pro-inflammatory stimuli, both acute and chronic, as well as its own cellular activation pattern which controls the intensity and time course of the inflammatory response.

3. The outside-in hypothesis and the adventitial signaling pathway

3.1. Signaling events after balloon angioplasty

The above discussion presents evidence that the adventitia is homeostatically regulated and responds to vascular injury from the outside-in through function as 1) the source

of migratory myofibroblasts responding to medial or intimal injury and 2) a depot for non-migratory fibroblasts and inflammatory cells that regulate the local adventitial environment, provide structural integrity to the entire vessel, and generate local humoral immune responses against injury. The first line of evidence for the outside-in hypothesis is that after vessel injury, but prior to established neointimal development, the adventitia and perivascular tissue become highly populated with neutrophils, macrophages and apoptotic cells [12,43]. Once the recruited cells are inside the adventitia, a number of genes and inflammatory molecules are expressed. Although it is not yet clear which chemotactic signals activate the adventitia during vessel injury and which subsequent signals secreted by the adventitia complete the wound healing process, a recent study found CCL2, also known as monocyte chemoattractant protein-1 (MCP-1), a target inflammatory molecule and potent leukocyte chemoattractant, highly expressed in myofibroblasts at the site of injury in the adventitial response (Fig. 1) [10]. CCL2 is known to induce macrophage recruitment and promote their subsequent activation. The authors also found temporal action and upregulation of other chemokine and chemokine-receptor expression in the adventitia after balloon angioplasty. Temporally, the cytokine CXCL2 was transiently upregulated in the adventitia at early time points less than 3 days and subsequently upregulated again in the neointima at 7 days, further supporting the progression of the inflammatory response from the adventitia toward the intima.

Other balloon angioplasty studies also confirm early adventitial action. Okamoto et al. found that within hours after angioplasty, P-selectin and VCAM-1 expression are upregulated in the endothelial cells of the vasa vasorum [44]. As early as 2 h after injury, neutrophils accumulate in the adventitia and perivascular tissues. Indeed at 14 days, adventitial VCAM-1 expression diminishes, while medial and neointimal VCAM-1 expression appears, providing additional evidence for the spatial propagation of the inflammatory response from the adventitia toward the intima.

The adventitial response to vessel homeostasis, like other pro-inflammatory events, appears to be redox sensitive as well. In healthy tissue the adventitia has been shown to regulate the oxidative state of the vessel wall by regulating the bioavailability of adventitially generated nitric oxide to the tunica media [45], most likely via increased production of superoxide anion [46]. It should be noted that reactive oxygen species (ROS) and chemoattractants, such as TGF-β and MCP-1, range in size up to 15 kD and therefore can easily diffuse through elastic laminae fenestrations which are approximately 2–7 μm in size [47]. In the balloon angioplasty model, perivascular delivery of a NADPH oxidase inhibitor, and subsequent reduction of adventitially derived ROS, reduced neointimal growth after injury of the rat carotid artery [48]. It was hypothesized that the lack of in vivo redox cues prevented the myofibroblast phenotypic switch into a migrating cell. This hypothesis was supported by in vitro studies demonstrating ROS able to induce the phenotypic switch from fibroblast into myofibroblast [48]. In addition, data from other studies support the ability of ROS to modulate the apoptotic regulation of cellular migration and proliferation that occurs during wound repair and remodeling [49,50]. Redox modulation may therefore, regulate the apoptotic death of the myofibroblast after its activation and migration to vascular injury.

Following the outside-in hypothesis, activated adventitial fibroblasts generate chemotactic signals in the form of cytokines and chemokines to recruit a milieu of inflammatory mediators from the perivascular tissue. Non-migratory adventitial cells modify the matrix composition and redox state of the local environment and simultaneously express surface adhesion makers, such as VCAM-1, to recruit other cell types including macrophages and lymphocytes. In this manner, the adventitia can affect the stability of the injured vessel, control the cellular infiltrate, and provide critical signals for the fibroblast to myofibroblast phenotypic switch. [47].

Also of importance are the distant effects of vascular inflammation which extend outside the vessel proper. Indeed multiple studies have observed that the inflammatory and proliferative response to balloon injury extends beyond the adventitia into the perivascular tissues, suggesting the potential for paracrine recruitment of leukocytes, lymphocytes, and other inflammatory mediators. Studies to control neointimal hyperplasia in the angioplasty model show that uptake of proteins and viral vectors into the medial layer is more effective when delivered perivascularly rather than via the lumen. Uptake of heparin [51], basic fibroblast growth factor [52,53], urokinase plasminogen activator [54], and viral constructs [55] into the vessel wall has also been shown to be more efficient when delivered perivascularly, indicating the involvement of the perivascular tissues and vasa vasorum for cellular traffic into and presumably out of the adventitia. Moreover, when whole aortic rings were transduced with endothelial nitric oxide synthase (eNOS), the vector preferentially partitioned into the adventitia [56]. It is also important to note that microvascular flow (as compared to bulk luminal flow) in the outside of the vessel may offer a favorable local environment for inflammatory cell recruitment, activation, and subsequent signaling.

4. The active adventitia: evidence from other models of vascular inflammation

Much of the data presented up to this point has been in the setting of balloon angioplasty wherein the adventitia is clearly injured and activated to elicit an inflammatory response to injury. However in most CVD states such clear adventitial provocation is absent. Diseases manifesting phenotypically in the intima and media, such as atherosclerosis, and those like arteriosclerosis and AAA that consume the entire artery clearly evoke the chicken-or-the-egg, i.e. which is stimulated first, the intima or the adventitia? It is clear that the complexity of CVD requires appreciation of both intimal and adventitial events as the outside-in and

inside-out responses coincide in various disease pathogenesis. Monocyte recruitment and migration through the endothelium is an important event in CVD and has indisputable clinical implications. However, based on recent studies in atherosclerosis it appears that outside-in mechanisms may have broad applicability in other vascular diseases [57–59].

4.1. Atherosclerosis

Strongly indicating that outside-in mechanisms favor the initiation of atherosclerotic disease, recent studies have shown increased vasa vasorum neovascularization [57,58] and macrophage presence [59] in early atherosclerotic lesions before plaque neovascularization. These hallmark studies indicate that in a hypercholesterolemic setting endothelial dysfunction in the lumen is preceded by increased vasa vasorum, creating a conduit for inflammatory cell transport into the vessel wall to promote chronic inflammation and plaque neovascularization. Furthermore, it is possible that endothelial dysfunction in the large vessel lumen may actually be a marker for the endothelial dysfunction taking place in the vasa vasorum. A thorough review of the role of the vasa vasorum in vascular inflammation is developed elsewhere in this issue (see *The Dynamic Vasa Vasorum* by Ritman and Lerman), however, the hallmark studies presented here highlight the applicability of the outside-in hypothesis to vascular atherosclerotic disease canonically considered an inside-out progression.

Studies have shown that after surgical removal of the vasa vasorum to promote adventitial ischemia the endothelium overlying the ressected areas becomes injured and the vessel ultimately becomes denuded [60,61]. In the setting of hyperlipidemia, however, atherosclerotic foam cell containing lesions form as a result of the dysfunctional adventitia beneath [60,62]. Ligation of intercostal arteries in order to restrict vasa vasorum flow to the adventitia of the aorta induced patchy intimal necrosis and cell loss in the areas with restricted vasa flow, further suggesting that the intimal endothelium is activated in part via the adventitia. [63]. While these studies suggest an anti-atherosclerotic role of the vasa vasorum in the setting of normal lipid levels, recent data from our laboratory corroborates the pro-atherosclerotic effect of the vasa vasorum in hyperlipidemic conditions. The descending aortas harvested from hyperlipidemic apolipoprotein E knockout mice treated with either granulocyte colony-stimulating factor or granulocyte macrophage colony-stimulating factor for 8 weeks demonstrated an increase in both adventitial vascularity and atherosclerotic lesion extent [64]. These data suggest that the adventitial vascularization may have a mechanistic role in inflammation of the aortic wall and subsequent atherosclerosis. It can be speculated then that the provocation for adventitial inflammation response in atherosclerosis is paracrine signaling from dysfunctional endothelium activated by lipoproteins, nutrient starved SMCs, and chronic inflammation. The adventitia responds in concert with the vasa vasorum by increasing vascularity, subsequently opening new and larger avenues through which lymphocytes and leukocytes can infiltrate. These studies underline the synergy between the entire blood vessel and the vasa vasorum. However, they also indicate the complexity of the pathophysiology in that disruption of perivascular blood flow can cause deleterious effects in the intima, whereas augmented vascularity is associated with increased vascular inflammation and atherosclerosis.

4.2. Hypertension

In models of hypertension, it has been shown that macrophage infiltration into the adventitia of large conduit arteries occurs in parallel with the development of hypertrophy of the vascular wall [8,65]. This occurs in the absence of significant recruitment of macrophages into the media or intima. Our group has proposed that mechanical strain imparted on the arterial wall as a result of an increase in blood pressure may be an important hypertensive signal. We have shown that the application of mechanical strain to cultured vascular smooth muscle cells results in an increase in MCP-1 expression [66]. Furthermore, we showed that in mice deficient in the MCP-1 receptor, CCR2, hypertension-induced adventitial macrophage infiltration of the arterial wall and subsequent vascular hypertrophy was significantly reduced [7]. These data suggest that there is physiologically relevant linkage between hypertension and macrophage infiltration of the adventitia and that macrophage infiltration of the adventitia is a necessary prerequisite for vascular hypertrophy.

4.3. Abdominal aortic aneurysm

AAA is characterized in part by an inflammatory response in the aortic wall that includes dramatic remodeling of the adventitia [67]. In light of this adventitial remodeling the tenets of the outside-in hypothesis are more easily illustrated. Indeed the pathogenesis of AAA has been shown to initiate with macrophage recruitment into the adventitia followed by subsequent presentation in the media [68]. In AAA, there are dramatic changes in the adventitia during development of the aneurysm with the adventitia thickening as the vessel wall expands. The adventitial becomes highly populated with neutrophils, macrophages, and lymphocytes. The adventitia of larger AAA also demonstrates increased expression of matrix metalloproteinase-3 (MMP-3) and MMP-9 [69,70]. A possible pathogenic mechanism of AAA formation was recently elegantly studied in a hyperlipidemic mouse model [5]. Supporting the outside-in hypothesis, the authors postulated that pro-inflammatory leukotrienes may impact CVD and AAA pathogenesis by decreasing MMP activity and ECM degrading enzymes that normally regulate vascular remodeling. Specifically they found that that 5-lipoxygenase pathway is active in the adventitia and that it may be involved in AAA formation via upregulation of plasma macrophage inflammatory protein-1α (MIP-1α).

Macrophages are presumably recruited into the adventitia, where they participate in the progressive aneurysmal inflammatory pathway.

It is clear that CVD pathologies progress in differential spatial and temporal manners, adding to the complexity of delineating mechanistic progression of the individual disease states. However, considering the outside-in hypothesis, we see that the basic mechanisms of cellular activation and migration are highly conserved across a variety of disease states and that the inflammatory events appear to initiate in the adventitia.

5. Discussion

The ability to develop new therapeutics and ultimately prevent vascular disease lies partially in our understanding of the critical signals and events involved in the associated inflammatory cascades of vascular remodeling. Traditionally considered an inside-out response, the current framework of vascular disease pathogenesis centers itself on the monocyte adhesion to the intima and the oxidative lipid hypotheses. We have presented data that lends consideration to a new outside-in hypothesis for vascular inflammation. In this hypothesis the inflammatory response is orchestrated from the adventitial side of the vessel. Resident adventitial fibroblasts respond to inflammatory signals by modulating normal homeostatic responses. A subpopulation of fibroblasts undergoes phenotypic switch into migratory myofibroblasts. These converted cells travel to the site of injured vasculature to mitigate vessel damage. At the same time non-migratory fibroblasts maintain the local adventitial environment during the inflammatory period. They simultaneously deposit additional collagen and matrix components, respond to and upregulate inflammatory chemokines and/or antigens, and regulate the local redox state of the adventitia. Clusters of lymphocytes reside in the vascular adventitia, some in arterial areas predisposed to disease. These B cells and T cells generate local humoral immune responses to produce antibodies against local antigen presentation by foam cells and antigen presenting cells. The cell populations work in concert to evoke an inflammatory response that propagates inward toward the intima.

It is clear that vascular inflammatory responses overlap among different vascular diseases. Vascular pathogenic mechanisms necessarily overlap in the same manner, conferring intimate interplay between the outside-in and the inside-out hypotheses. Formation of early atheromata on the surface of the intima minimally affects, if at all, the pathology of the adventitia below, but at the same time can signal neovascularization of the neighboring vasa vasorum. These lesions presumably develop under the tenets of monocyte homing to an injury endothelium and formation of the superficial lipid containing lesion and may generate paracrine signals to activate the adventitia at later atherosclerotic stages. In contrast, at the earliest stages of development AAA show dramatic changes in medial and adventitial pathology occurring around an intact intima and open lumen. It is likely that vascular pathology is ultimately mediated by complex mechanisms originated from both the intimal and adventitial surfaces of the arterial tree. Ultimately, the ability to delineate how inflammation affects vessel homeostasis lies in part by considering the adventitia as an active component of the arterial wall that confers structurally integrity as well as a source of inflammatory mediators.

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