Frontiers in Nephrology: Early Atherosclerosis—A View Beyond the Lumen

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ABSTRACT

Endothelial dysfunction is an established clinical marker of early coronary artery disease and has been shown to be associated with increased cardiovascular morbidity and mortality. New concepts now extend the view of endothelial dysfunction beyond the traditional involvement of the coronary arterial endothelium alone. Recent research indicates that the coronary vessel wall, especially the vasa vasorum, as well as bone marrow–derived endothelial progenitor cells may be subject to proatherosclerotic changes, even before the development of angiographically evident endothelial dysfunction; therefore, "microvascular endothelial dysfunction," which is composed of dysfunction of the vasa vasorum's endothelium as well as "microcellular endothelial dysfunction," reflecting impaired mobilization and function of endothelial progenitor cells, may precede "macrovascular endothelial dysfunction." Vasa vasorum neovascularization, with endothelial leakage and dysfunction increasing influx of proinflammatory and proatherogenic cellular and noncellular substances into the vessel wall, is proposed as one feature of this new concept. In addition, the role of bone marrow–derived endothelial progenitor cells is discussed as are the potential impact of impaired progenitor cell mobilization, release from the marrow, and function in acute and stable coronary artery disease. Finally, potential future therapies are proposed, focusing on interventions that may prevent or diminish the development of the microvascular and microcellular endothelial dysfunction.


THE TRADITIONAL VIEW OF ATHEROSCLEROSIS

Atherosclerosis is considered a systemic, chronic inflammatory disease predominantly of the arterial vessel wall with local complications that determine morbidity and mortality. The classic response-to-injury model described physical injury to the endothelium as the first step of atherogenesis, but it has been subsequently recognized that endothelial dysfunction (e.g., the impaired bioavailability of the endothelial-derived vasodilator substance nitric oxide [NO]) may also be considered as a functional “injury.” Numerous studies have identified risk factors for the development of endothelial dysfunction, but factors such as hypercholesterolemia, smoking, diabetes, metabolic syndrome, and hypertension are the most prominent.

Endothelial dysfunction involves the trans-formation of vasoprotective properties of the endothelium into proatherosclerotic features: The endothelium becomes procoagulant, produces vasoconstrictive molecules, and releases cytokines that attract inflammatory cells, including lymphocytes and macrophages. Accumulation of these inflammatory cells, as well as increased leakiness (i.e., permeability) of the endothelium, eventually leads to formation of histologically identifiable atherosclerotic lesions. At this point, atherogenesis may become a self-perpetuating process, a vicious cycle in which accumulating inflammatory and noninflammatory cells release chemokines, cytokines, and growth and transcription factors that attract more inflammatory cells and lead to progression of the atherosclerotic lesion and eventually to local complications, namely plaque rupture with local arterial thrombosis (Figure 1).

Recent research has broadened our view of the atherosclerotic process. Whereas the luminal endothelium was traditionally considered as playing the main role in vascular regulations, we know today that a considerable amount of endothelium is also present in the abluminal part of the vessel wall, at the level of the vasa vasorum. Several studies identified a possible major impact of inflammatory invasion from the adventitia into the inner vessel wall layers, rather than only the entrance via the luminal endothelium.

It is therefore obvious that the vascular endothelium and its interaction with the surrounding environment play a crucial role in the pathogenesis of athero-
sclerosis. Exposed to multiple circulating atherogenic risk factors, it undergoes a constant injury-and-repair process that may result in several potential outcomes, which may also be a progressive cascade of events (Figure 1):

1. The repair is successful and heals the endothelium and vascular wall, and endothelial function remains normal.
2. The repair is unsuccessful, and although the endothelium and vascular wall show no morphologic changes, endothelial dysfunction occurs (impaired bioavailability of NO, abnormal vasoconstriction).
3. The repair is unsuccessful, results in vascular scarring characterized by fibrotic tissue and calcification, and promotes the injury process.
4. The repair is lacking or insufficient, and the injury process continues into complicated atherosclerotic lesions.

The proper function of all systems involved in the repair process as well as their adequate interaction is crucial for a successful repair. Endothelial dysfunction has been identified as a marker of early atherosclerotic disease before the development of a lumen-compromising plaque and, hence, as an indicator of the unsuccessful repair process. As a result, endothelial dysfunction has been shown to be associated with adverse cardiovascular outcomes; however, endothelial dysfunction not only may be the result of unsuccessful repair but also may actually be among the underlying causes. Indeed, Lerman and Zeiher recently suggested that the site of endothelial dysfunction should be extended beyond the traditional location at the conduit vessels into the vessel wall and possibly even into the bone marrow, the origin of endothelial progenitor cells (EPC). In addition, EPC have been demonstrated to play an important role in the repair of injured endothelium, and EPC dysfunction may be a very early marker of atherosclerotic disease.

The pathomechanisms of conduit vessel endothelial dysfunction have been relatively well established. Therefore, in this review, we focus on possible expression and consequences of endothelial dysfunction within the vessel wall and EPC, two novel and exciting areas of current basic and clinical research that may open new ways for therapeutic approaches.

**MICROVASCULAR ENDOTHELIAL DYSFUNCTION OF THE VASA VASORUM**

Early endothelial injury (e.g., in hypercholesterolemia, smoking, or diabetes)
may lead to a leaky endothelial layer. This early injury not only takes place at the luminal endothelium but also may conceivably extend to the endothelium of the vasa vasorum within the vessel wall. Vasa vasorum (both arterial and venous) are microvessels located within the wall of bigger arteries, such as the renal and coronary arteries, providing blood supply to and drainage from the vessel wall. Importantly, the arterial vasa vasorum originate either from the artery itself (vasa vasorum interna) or from its major branches (vasa vasorum externa) and are thus directly connected to the systemic circulation (Figure 2). The significance of the vasa vasorum becomes evident when considering the endothelial exchange surface that they provide within the vessel wall, which is as large as 33% of the main lumen’s endothelium in normal arteries. Several recent studies demonstrated that the vasa vasorum indeed serve as important entry ports for the influx of cellular and noncellular proinflammatory and proatherosclerotic substances (e.g., oxidized LDL, cytokines, macrophages) into the vessel wall and that they in turn also play a significant role in draining the arterial vessel wall. Moreover, it has been shown in a porcine model of hypercholesterolemic early atherosclerosis that vasa vasorum neovascularization occurs before the development of atherosclerotic plaques. This vasa vasorum neovascularization leads to an average increase of endothelial exchange surface within the vessel wall of ≥60%, which conceivably further promotes influx of proatherogenic substances into the vessel wall. In addition, using cryogenic microcomputed tomography, we demonstrated that the drainage function of these newly formed vasa vasorum is also significantly impaired in this animal model, leading to saturation of the vessel wall tissue and longer exposure time of medial and adventitial cells to proinflammatory and proatherogenic substances. Furthermore, functional data suggest that in the porcine animal model, hypercholesterolemia is associated not only with macrovascular endothelial dysfunction (i.e., paradoxic vasoconstriction) but also with dysfunction of the vasa vasorum’s endothelium. This may lead to intermittent vasoconstriction causing hypoxia within the vessel wall and subsequent reactive, local production of vascular endothelial growth factor (VEGF) with enhanced vasa vasorum neovascularization and a further increase in endothelial exchange surface, creating a vicious cycle.

Together, these important facts suggest that the inner and the outer arterial vessel wall layers are simultaneously exposed to proatherosclerotic cellular and noncellular blood components. In fact, the medial layers are literally surrounded by the influx of blood-born noxious substances or cells through the endothelium of both the main lumen and the vasa vasorum. This indicates that microvascular endothelial dysfunction at the level of vasa vasorum in the early phases of atherogenesis may amplify alterations in endothelial exchange surface.

Figure 2. Volume-rendered microcomputed tomography images of the two types of arterial coronary vasa vasorum (examples are from porcine coronaries that represent human anatomy). The main coronary artery lumen and major branches are displayed in gray, vasa vasorum in yellow. Vasa vasorum interna (left) arise directly from the main coronary artery lumen and arborize into the vessel wall. Vasa vasorum externa (the most prevalent type of arterial vasa vasorum; right) originate from major branches of the coronary artery and dive back into the main coronary’s vessel wall.
within the vessel wall that disrupt its homeostasis and promote atherogenesis.

It is not surprising, then, that increasing evidence suggests that these “vessels of vessels” (vasa vasorum) play an important role in the initiation, progression, and complications of atherosclerosis. Moreover, the spatial vasa vasorum density and distribution in different vascular beds (carotid, coronary, renal, and femoral arteries) are associated with their propensity to develop atherosclerosis.

In summary, early microvascular endothelial dysfunction has a significant impact on vessel wall homeostasis, leading to a proatherosclerotic environment even before the development of visible atherosclerotic lesions. The question whether vasa vasorum neovascularization is beneficial or detrimental, whether it is a trigger or just an epiphenomenon of atherosclerosis, is still not fully answered; however, it is evident that the severity of atherosclerosis correlates with vasa vasorum neovascularization, and animal models of atherosclerosis indicate that inhibition of vasa vasorum neovascularization reduces plaque size and influx of proinflammatory cells. Clearly, vasa vasorum may be a very interesting future therapeutic target.

**MICROCELLULAR ENDOTHELIAL DYSFUNCTION OF EPC**

Bone marrow–derived EPC generated substantial interest and have been the focus of basic and clinical cardiovascular research in recent years. Emerging evidence suggests that EPC play an important role in the repair of endothelial injury and restoring local endothelial function. Conversely, a reduction of EPC numbers has been shown to be associated with endothelial dysfunction and adverse cardiovascular outcomes.

In stable coronary artery disease (confirmed by coronary angiography), a low number of EPC (CD34+/VEGFR-2+) is associated with a higher risk for cardiovascular death, first major cardiovascular event, revascularization, hospitalization, and shorter cumulative event-free survival. In contrast, in patients with acute coronary syndromes (acute myocardial infarction and unstable angina pectoris), the majority of published literature suggests that the number of EPC is increased. This increase has been observed within a few hours from the onset of the acute event until 2 mo after. Most likely, the acute increase in EPC mobilization is caused by concomitant release of inflammatory and hematopoietic cytokines (e.g., VEGF, IL-8, stromal-derived factor-1). Alas, what looks like a reasonable, clear difference of EPC counts in stable (low EPC) versus unstable (high EPC) coronary syndromes secondary to a release of mobilizing cytokines during the stress situation was lately challenged by a study by Guven et al., who found that in patients with angiographically documented significant coronary artery disease, the number of EPC was actually increased and EPC numbers correlated with maximum angiographic stenosis severity. However, this study used more rigorous histologic and flow cytometric techniques to quantify EPC as well as an angiographic definition of coronary artery disease, which makes it hard to compare directly with earlier, potentially conflicting studies.

In all observations, however, it is crucial to exclude potential cofounders that can influence the EPC counts. These include comorbidities, current medical therapy, and the interval between acute stress events (e.g., acute myocardial infarction, unstable angina pectoris, other significant medical conditions) and previous coronary interventions. Furthermore, is seems that potentially conflicting data may be due at least in part to methodologic differences, especially regarding the definition of EPC and coronary artery disease. Future studies will have to clarify the role of the various types of EPC in well-defined clinical scenarios, especially because EPC may become a future therapeutic target.

Despite the ongoing controversy about how best to classify EPC, identification of hematopoietic cell surface markers such as CD34 and CD133 and the vascular endothelial growth factor receptor (VEGF-R2/KDR) is the current standard. In addition, recent studies have suggested an important distinction between EPC purely identified by cell surface antigens or by their capability to form discrete colonies of endothelial cells in culture (CF-EPC). Hence, a reduction in the number of circulating EPC may not necessarily reflect in reduced potential for neovascularization or re-endothelialization.

Studies in patients with diabetes have shown that recruitment of EPC to the vascular injury site is impaired, indicating that endothelial dysfunction, which is well documented in patients with diabetes, may play a role in this scenario. In addition, animal models of decreased NO activity show impaired functionality of EPC in repairing the vascular injury site. Another potential role for the EPC from mesenchymal origin may be in determining the kind of vascular repair such as coronary calcification, with EPC that carry an osteogenic potential.

The extension of endothelial dysfunction may be seen in two distinct ways. On the one hand it may extend into bone marrow microvascular endothelial dysfunction (i.e., the release of EPC from the bone marrow is impaired as a result of loss of efferent vessels). On the other hand, cardiovascular risk factors and aging may lead to impaired mobilization, migration, proliferation, and survival of EPC, as well as to dysfunctional EPC that are thus proatherogenic. Of course, both mechanisms may coexist; therefore, endothelial dysfunction and the abnormal repair may also be characterized by the modification of the number and function of proatherogenic EPC produced and released into systemic circulation; therefore, both the vessel wall (vasa vasorum) and circulating EPC are potentially impaired in the setting of endothelial dysfunction. Moreover, these two systems might be related. The discrepancy between the number of circulating EPC and their colony-forming capacity raises the hypothesis that reduction of peripheral EPC may reflect the impairment of re-endothelialization at the injury site, whereas the increase in the fraction of CF-EPC may confer increased...
propensity for neovascularization that may exhibit in vasa vasorum. Whereas vasa vasorum neovascularization may be detrimental as a result of the increased number of entry ports for proatherosclerotic substances into the vessel wall, re-endothelialization of the vascular injury site with EPC would be beneficial. Hence, the observations of reduced EPC in coronary artery disease patients in one study\(^4\) but increased colony-forming EPC in another\(^5\) might not be a true contradiction. Whereas the former explains the failure to repair the injured endothelium or alternatively re-endothelialization with inadequate EPC (e.g., with osteogenic potential or other dysfunctions), the latter explains vasa vasorum neovascularization associated with progression of atherosclerosis.

It is obvious that the atherosclerotic process is very complex and affected by the interactions among many systems; therefore, vasa vasorum may well represent the tubes that deliver the progenitor cells into the vessel wall and their neovascularization may enhance delivery.\(^1\) Vasa vasorum neovascularization may also be detrimental if this facilitates delivery of proatherogenic EPC to the injured site.

**POSSIBLE FUTURE THERAPEUTIC APPROACHES**

Clearly, future antiatherosclerotic therapy has to start very early, before functional and morphologic changes that take place potentially become irreversible. In patients with cardiovascular risk factors, local or systemic delivery of endogenous EPC may be an option; however, this approach has to be carefully evaluated because recent research indicates that transfused EPC may promote atherosclerosis\(^2\) potentially by their paracrine function to attract proatherosclerotic cells as well as by induction of neovascularization. In addition, they may differentiate into smooth muscle cells as a result of a potential common mesenchymal stem cell precursor\(^3\) and thus promote lesion progression. Induction of vessel wall neovascularization by EPC may also be detrimental in a patients with existing complex lesions; plaque rupture and acute lethal thrombosis may be a consequence.\(^4\) Conversely, experimental data have demonstrated that intravenous transfusion of EPC may reduce neointima formation.\(^5\)

The same complexity is true for a potential antiangiogenic treatment to prevent vasa vasorum neovascularization. The current data are equivocal,\(^6\) and the response to therapy may depend on the vascular beds involved. One possible solution might be local, specifically designed drug delivery (e.g., through resolvable stents\(^7\)) that may not have long-term adverse effects.

There is an increasing body of evidence demonstrating the pleiotropic effects of hydroxymethyl glutaryl CoA reductase inhibitors (statins); especially their anti-inflammatory and antiangiogenic effects certainly make this a possible approach.\(^8\) In addition, several studies have shown that statins increase the mobilization of bone marrow–derived EPC, possibly through the Akt signaling pathway.\(^9\) This increase is reported to be comparable to the effect of VEGF on EPC mobilization. Taking into account the low prevalence of adverse effects, several investigations are advocating the concept of a wider use of statins, considering, of course, that an increase of circulating EPC would be beneficial. This concept is underscored by the recent placement of statins over the counter in the United Kingdom. The questions are the potential long-term adverse effects and ethical boundaries that may prevent us from exploring potential interventions.

It seems that the future of antiatherosclerosis therapy lies in the very early detection of typical features of atherosclerosis and endothelial dysfunction (inflammation, neovascularization); unfortunately, these are difficult to detect using current in vivo imaging techniques. In addition, biomarkers are evaluated for the detection of patients at risk, but larger, prospective studies are still needed to pinpoint the best measurements that may allow us to identify the patient with coronary artery disease before he or she develops endothelial dysfunction of any kind.

New diagnostic techniques that are designed to detect the early manifestation of atherosclerosis are needed. These new diagnostic techniques should include the assessment of endothelial function, the number and function of EPC, and the degree of inflammation and neovascularization. On the basis of these novel diagnostic tests, a new therapy that is directed toward the mechanism of atherosclerosis in addition to the correction of risk factors may attenuate disease progression and complications.

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**DISCLOSURES**

None.

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