

## Atherosclerosis 2005 Recent Discoveries and Novel Hypotheses

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**A**therosclerosis is an inflammatory process that selectively affects arteries and is highly prevalent in both women and men. Thrombo-occlusive complications of atherosclerosis, including stroke and myocardial infarction, are major causes of morbidity and mortality. Senescence of the arterial tree is a biological process that follows a natural course that can be accelerated by the combined injury of multiple risk factors. A repair capacity that is intrinsic to the organism can counteract the natural time- and risk factor-dependent obsolescence of arteries. The failure of this repair process is intimately linked to atherosclerotic inflammation and lesion formation. Recruitment and targeting of bone marrow-derived progenitor vascular cells, and probably vascular progenitors originating in other tissues, that have an intrinsic capacity for repair of the vascular wall represent a promising area for research and development.

This review focuses on recent discoveries relevant to vascular progenitor cells and others that are likely to influence atherosclerosis research. It highlights the exciting recent findings that demonstrate the vital role of vascular progenitor cells in the homeostasis of the vessel wall and the mitigation of atherosclerosis. In the context of atherosclerosis, this review also discusses the relevance of this paradigm to pharmacological treatment of atherosclerosis and the process of aging.

### Of Risk Factors and Atherosclerosis

The entire arterial tree is exposed to the risk factors for atherosclerosis, yet lesions of atherosclerosis have a distribution along arteries that is not uniform and are strongly influenced by hemodynamic factors (Figure 1).<sup>1–5</sup> Atherosclerosis develops as a result of sustained injury to the arterial wall, and several culprits have been identified.<sup>6–11</sup> Importantly, an elevated cholesterol level, particularly LDL cholesterol, is a risk factor for atherosclerosis and resulting thromboembolic complications.<sup>7,8</sup> Data in humans indicate that even an increase in total cholesterol from 150 mg/dL (3.9 mmol/L) to no more than 200 mg/dL (5.2 mmol/L) could contribute to cardiovascular death.<sup>12</sup> Instructively, many mammals, including laboratory mice fed a regular chow, display a

total cholesterol well below 150 mg/dL (3.9 mmol/L), but when their diet is Westernized, their cholesterol rises to  $\geq 200$  mg/dL (5.2 mmol/L), as it does for humans.<sup>13</sup> Incremental reduction in LDL cholesterol with statin drugs to a target level  $< 100$  mg/dL (2.6 mmol/L) improves outcome for patients with coronary artery disease.<sup>14,15</sup> In light of these observations, the previously accepted targets for total cholesterol (200 mg/dL or 5.2 mmol/L) and LDL cholesterol (100 mg/dL or 2.6 mmol/L) might need to be revisited.<sup>12,14,15</sup> Other factors that can lead to vascular injury include infectious agents, excessive blood pressure, inflammatory mediators, and glycation events, all involving accrued oxidative stress.<sup>10,11</sup> Although most of these factors apply homogeneously to the arterial wall, atherosclerosis develops as a mosaic (Figure 1), strictly influenced in its topography by hemodynamic modifiers.<sup>1,2</sup> The specific mechanism for such distribution remains largely uncharacterized, but arterial hemodynamics can affect vascular cell signaling and gene expression.<sup>16</sup>

Repair and remodeling of the arterial wall are regulated by a myriad of local events and stimuli. When remodeling becomes pathological, especially in advanced atherosclerosis, it can lead to reduced flow as a result of narrowing of the lumen and consequent ischemia for downstream tissues. Local injury of the arterial wall triggers a powerful recruitment of vascular cells, either through in situ proliferation or by engraftment of bone marrow-derived progenitor cells.<sup>17,18</sup> Local availability and activity of mitogens, differentiation factors, cytokines/chemokines, adhesion molecules, proteases, matrix components, and vasoactive agents can affect this repair process. Furthermore, the local impact of primary and secondary blood flow currents may also affect the efficacy of a repair process that requires the successful engraftment of progenitor cells to the arterial wall.

### Response to Injury: Local Versus Central Process

The injured arterial wall, in addition to the local response, mounts a profound systemic response that involves a cascade of molecular and cellular events to promote the repair of the vessel wall.<sup>19–21</sup> This response includes the production of cytokines and growth factors; the release and circulation of

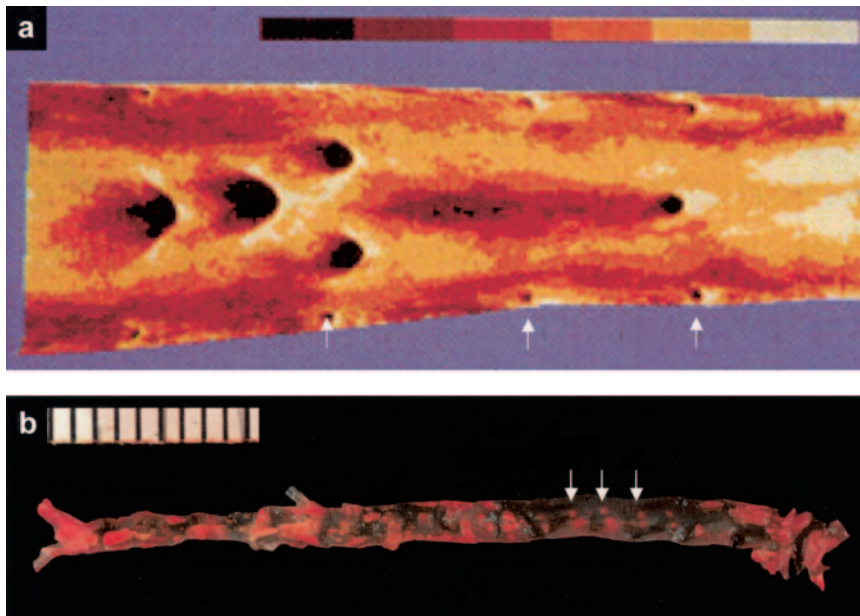
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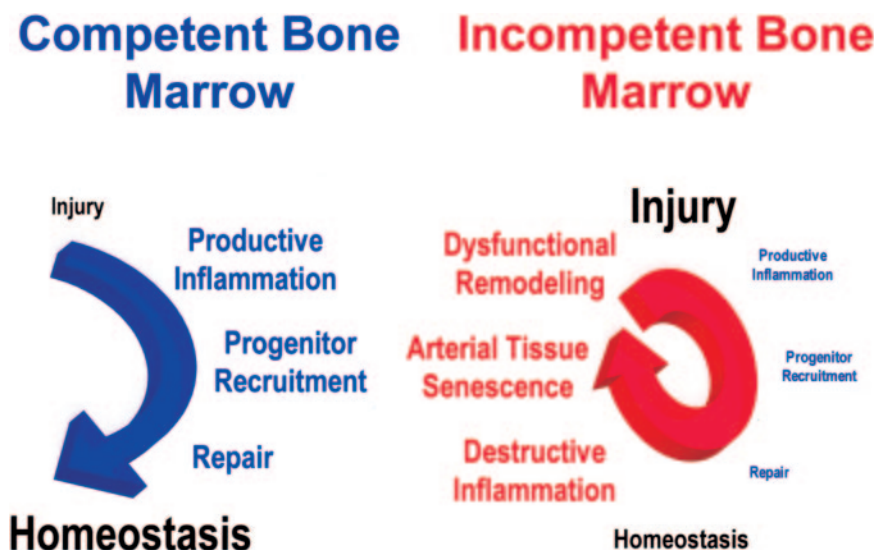


**Figure 1.** Mosaic distribution of atherosclerotic lesions. The distribution of lesions of atherosclerosis is not monotonous across the arterial tree. Instead, lesions have a tendency to develop earlier in areas of increased hemodynamic stress (eg, branching arteries). Evidence in support of this concept is found in both human (a) and murine (b) atherosclerosis. a, Compilation of atherosclerosis distribution within the human abdominal aorta of >2000 specimens collected postmortem in individuals who had died of noncardiac causes before 33 years of age. Atherosclerosis was detected with Sudan IV staining, and the color scale indicates the frequency at which lesions are found at each site (light color indicates low frequency [ $<10\%$ ]; dark color indicates high frequency [ $>50\%$ ]). Adapted from McGill HC Jr, et al. *Arterioscler Thromb Vasc Biol*. 2000;20:836–845, with permission. b, Distribution of atherosclerotic lesions in the mouse aorta (single sample) in which lesions were stained also with Sudan IV. Note that in both cases, lesions develop in area of branching vessels. In both images, the proximal end of the artery is on the right.

progenitor cells by the bone marrow and other organs; the anchorage of progenitors to the arterial wall; the integration of the progenitors into a rejuvenated endothelium; and autocrine, paracrine, and endocrine regulation of the arterial wall by recruited progenitors and the repaired endothelium. This process can be misguided, however, and can contribute to vascular lesion progression. Inflammatory cytokines, growth factors, and other messengers are produced by the injured vascular wall, and some of these signals can be amplified through biological relays.<sup>22,23</sup> The triggered response varies markedly, according to local and systemic capacity of the surrounding organism for arterial repair.<sup>24–26</sup> As a general rule, a rapid and successful repair of the arterial wall in response to growth factor and cytokine production is required to contain the inflammatory reaction within physiological limits.<sup>24</sup> Failure to repair the arterial wall in response to

inflammatory signals will further promote atherogenesis and hence create a positive feedback loop that propagates vessel injury<sup>24–26</sup> (Figure 2).

Such imbalance between capacity for repair and inflammatory agonists promotes atherosclerosis. For example, the concentration of proinflammatory cytokines circulating in the vessels of an overweight patient may be markedly higher than in an individual with optimal body weight<sup>27</sup> owing to the enhancing effect of the adipose tissue on the production of cytokines such as interleukin-6. Recent studies on infectious agents such as chlamydia and cytomegalovirus suggest that they may contribute to the exaggeration of the inflammatory process by promoting further deleterious changes at the level of the arterial wall.<sup>11</sup> In contrast, it has been suggested that elevation of selected lipoproteins such as HDL and, in particular, the Milano mutant of HDL has the potential to



**Figure 2.** Mechanistic link between arterial repair and inflammation. In the presence of competent bone marrow, an arterial injury triggers an inflammatory reaction that can be seen as constructive, or self-limited, because it serves to trigger the production and release by the bone marrow and perhaps other tissues of vascular progenitor cells capable of arterial repair. This inflammatory reaction is self-contained because the repair of the arterial wall results in the discontinuation of the stimulus that had triggered the inflammatory reaction, although some distant processes may help sustain inflammation despite successful repair. In contrast, in the absence of competent marrow, an arterial lesion has no opportunity to become repaired, and this lack of repair contributes to perpetuating and even intensifying of the atherosclerotic inflammation (destructive inflammation, leading to a positive feedback loop vis-à-vis inflammation), with progressive senescence and dysfunctional remodeling of the arterial wall.

lower inflammation and consequent vascular injury.<sup>28,29</sup> Of particular interest is the capacity of HDL to retard the progression of atherosclerosis without marked modification of inflammatory markers.<sup>29</sup> The mechanism that mediates the beneficial effect of HDL on atherosclerosis is mostly uncharacterized but appears to involve reverse cholesterol trafficking.

Although circulating cytokines and growth factors can lead to accelerated inflammation of the injured vascular wall, it was recognized only recently that such molecules are not necessarily noxious and can, in fact, promote the productive recruitment of bone marrow–derived mononuclear cells.<sup>24–26</sup> This observation led to the concept of “constructive” (self-limited, to the extent that it promotes tissue repair) versus “destructive” (further contributes to tissue damage) inflammation (Figure 2). In particular, vascular progenitor cells, especially endothelial progenitor cells (EPCs), with intrinsic capacity for repair of the vascular wall are important contributors to the vascular repair process.<sup>24–26,30,31</sup> Such cells are produced and released by the marrow and perhaps other tissues harboring progenitor cells in response to signals derived from the injured vascular wall and anchor at the level of arterial lesions.<sup>24</sup> Evidence for their contribution to the repair of the arterial wall is emerging. Their absence or dysfunction results in more advanced vascular lesions, whereas their administration to animals deprived of competent vascular progenitor cells can retard the onset of vascular disorders.<sup>24–26</sup> Understanding the production of competent cells involved in the repair of the arterial wall and arising from a distant site (bone marrow and elsewhere) represents a potentially important opportunity for advancing the understanding of vascular disorders, their prevention, and/or their cure.

### **Aging in the Presence of Risk Factors and Atherosclerosis**

Arterial repair can be successful and complete, in which case the inflammatory reaction associated with vascular injury may resolve and markers of inflammation may vanish from the circulation. In many situations, however, the repair is incomplete and/or pathological and leads to remodeling of the arterial (or venous) wall. For some patients, the remodeling of the vascular wall may be discrete so that blood flow remains unperturbed, whereas for others, remodeling may lead to pathological enlarging or narrowing of the lumen with or without thromboembolic complications. Pathological remodeling can lead to aneurysm formation, stenosis, restenosis, or dissection of arteries. Aging, in the presence of risk factors for vascular disease, can lead to depletion of bone marrow cells capable of repair of the arterial wall.<sup>24–26</sup> Indeed, the depletion of bone marrow–derived progenitor cells may account for a large fraction of the aging risk in the development of vascular disorders.<sup>26</sup> Such depletion of competent cells may involve the loss of production of progenitors by a dysfunctional marrow or the marrow production of dysfunctional progenitor cells that are therefore incapable of repair.<sup>26</sup> Competent progenitors might also be produced but may not be released by the marrow. It is further possible that repair-competent progenitor cells are produced and circulate but are

unable to latch onto arterial lesions or that, once latched, these competent cells might be unable to mount a successful repair process as a result of local hemodynamic and other factors.

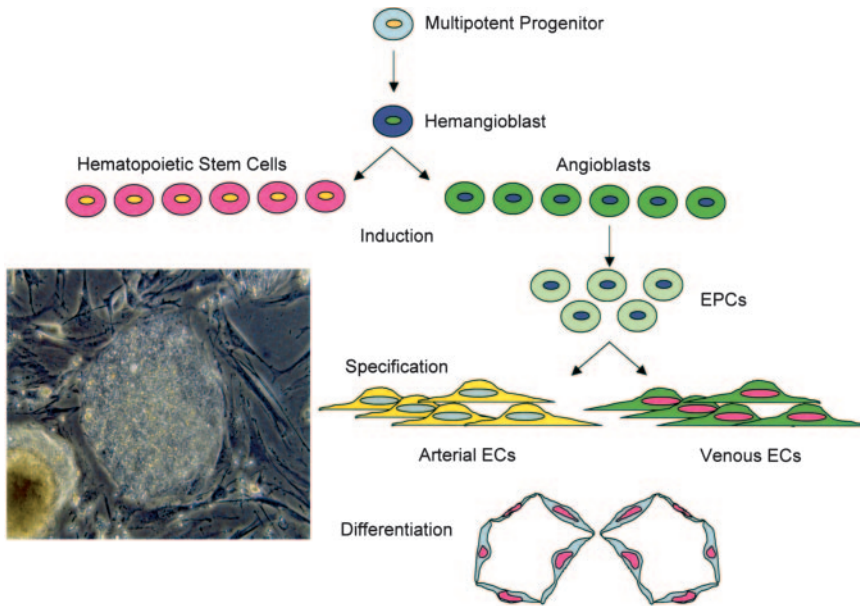
Risk factors, independently of aging, have also been shown to be associated with lower circulating EPC counts.<sup>32–34</sup> Whether such risks affect the bone marrow production of EPCs directly or promotes the exaggerated consumption of circulating EPCs or a combination of the 2 processes remains to be defined. In the case of some risk factors, particularly diabetes mellitus, there is little doubt that the biology of the marrow itself is impaired.<sup>35,36</sup> EPCs may also be destroyed, as in certain autoimmune disorders, and thus unable to complete their repair mission.

### **Of Progenitor Cell Transfer and Atherosclerosis**

Intravenous transfer of competent bone marrow from wild-type or young apolipoprotein E knockout (ApoE<sup>−/−</sup>) to ApoE<sup>−/−</sup> recipient mice fed a fat-rich diet prevents the development of atherosclerotic lesions.<sup>24</sup> In contrast, administration of bone marrow from older ApoE<sup>−/−</sup> mice is of limited benefit.<sup>24</sup> Goldschmidt-Clermont and coworkers observed differences in the composition of the marrow of young mice versus older ApoE<sup>−/−</sup> mice and, in particular, a reduction in cells displaying typical characteristics of EPCs in the marrow of old ApoE<sup>−/−</sup> mice.<sup>24</sup>

Markers of inflammation (C-reactive protein, interleukin-6 [IL-6], or vascular endothelial growth factor [VEGF]) identify patients at increased risk for cardiovascular thromboembolic events<sup>37</sup>. In ApoE<sup>−/−</sup> mice fed a fat-rich diet, chronic hyperlipidemia is associated with elevation of IL-6 and VEGF.<sup>24</sup> Intravenous administration of competent bone marrow to these mice resulted in marked and sustained suppression of the blood levels of these markers of inflammation.<sup>24</sup> Intravenous administration of bone marrow cells originating from older ApoE<sup>−/−</sup> mice (6 months old) had only a limited and transient effect on IL-6 and increased, rather than decreased, the level of VEGF. Hence, these experiments supported the following concepts. First, the absence of “competent” bone marrow may contribute to the progression of atherosclerosis in the presence of risk factors such as hyperlipidemia; as a corollary, supplementing mice at risk for developing atherosclerosis as a result of hyperlipidemia with competent bone marrow cells delivered intravenously can restore arterial repair and prevent lesion development. Second, there is an intriguing relationship between bone marrow progenitors and inflammation (Figure 2) so that in mice exposed to a significant risk factor like hyperlipidemia, the levels of blood markers of inflammation are elevated. Once they are supplemented with competent bone marrow cells, however, inflammation subsides, thus halting a cascade of events that ultimately lead to atherosclerotic plaque formation. In turn, the successful repair of arteries and putative correction of bone marrow abnormalities may eliminate the stimuli for production of inflammatory cytokines. Instructively, the administration of competent bone marrow cells normalized inflammatory markers in the absence of improvement in risk factors (the total cholesterol level in the ApoE<sup>−/−</sup> mice remained >1200 mg/dL). Hence, according to the latter





**Figure 3.** Differentiation pathway for EPCs within the adult marrow. The effect of aging in the presence of risk factors could be taking place at several steps in the differentiation pathway of EPCs, resulting in obsolescence. Several steps probably require the reorganization of the chromatin of progenitors via epigenetic mechanisms. The close interaction between progenitors, stromal cells, and extracellular matrix proteins (inset) has a modifier impact on the effect of growth factors and cytokines on the production, differentiation, and release of the vascular progenitors, particularly EPCs. A better understanding of these mechanisms creates a new opening for therapeutic intervention on bone marrow senescence and consequent atherosclerosis.

scenario, an important feedback loop exists between successful repair of arteries and suppression of systemic inflammation in situations of atherosclerosis.<sup>26</sup> This impact of a competent repair process does not necessarily apply to inflammation that develops independently of the presence of arterial lesions.

Many questions related to arterial repair such as the specific identity of the cells that contribute to the repair of vascular lesions remain unanswered. Transient culture of the cells extracted from the marrow may enhance their repair capacity through an unknown mechanism. The partial depletion of EPCs found in the marrow of older ApoE<sup>-/-</sup> mice suggests that these progenitors could be at play for successful repair.<sup>24</sup> It remains unknown why aging in the presence of severe risk leads to depletion of competent progenitors. One possibility is that the aging progenitors are becoming senescent. Partial senescence is an important process that allows tissues such as the cardiovascular system to escape malignant transformation by keeping uncontrolled proliferation in check. The maintenance of a population of competent progenitors within the bone marrow niche (which probably has intrinsic ability to curtail malignant transformation) is crucial for maintaining the integrity of the repair capacity of the organism toward the arterial tree. The obsolescence of the marrow and consequent depletion of vascular progenitors are of grave consequence in the maintenance of arterial homeostasis.

Thus, an intriguing opportunity has emerged from the concept of bone marrow senescence that consists of resurrecting the ability of bone marrow-derived vascular progenitors to repair damaged arterial walls. In this context, genetic or chemical modifications of EPCs resulting in the activation of the Akt pathway improve the survival and reduce the senescence of the progenitors cells.<sup>38-41</sup> Inhibitors of HMG-CoA reductase, for instance, have been shown to enhance the proliferation, migration, and survival of EPCs.<sup>38,41</sup> A putative mechanism for this involves the activation of the phosphatidylinositol 3-kinase (PI 3-kinase) and protein kinase B (Akt)

pathway,<sup>38,39,41</sup> a signaling pathway known to regulate downstream nitric oxide production and cellular survival. Importantly, endothelial nitric oxide synthase has recently been shown to play a regulatory role in the recruitment and mobilization of bone marrow-derived progenitor cells.<sup>42</sup> Additionally, the ability of the PI 3-kinase-Akt pathway to transcriptionally and posttranscriptionally regulate cell cycle progression in EPCs might play a role. Recent evidence indicates that atorvastatin could reduce senescence of EPCs by increasing the expression of cell cycle-promoting proteins while reducing the cell cycle-inhibitory protein p27. Reduced senescence might counter the effect of aging and enable progenitor cells to continue with their reparative functions.

It is also likely that additional types of vascular progenitors and perhaps nonprogenitor bone marrow cells contribute to the repair process, whereas other cells of the marrow may have an opposite effect. In particular, inflammatory cells may have a detrimental effect on atherosclerotic lesions according to our theory of constructive, self-limited inflammation versus destructive inflammation (Figure 2).<sup>43,44</sup>

Furthermore, does the repair of various sections of the vascular tree involve a generic type of vascular progenitor cells, or are there specific progenitors for the repair of each section of blood vessels (ie, small versus large versus pulmonary arteries)? In the context of this question, it is relevant to note that the panoply of expressed genes varies markedly among endothelial cells derived from various aspects of the arterial tree,<sup>45</sup> perhaps as a consequence of variable local hemodynamic parameters. Alternatively, it is tempting to speculate that such differences in phenotype could be preconditioned at the level of EPCs that latch onto various vascular targets, phenotypic differences that could be imprinted by factors relevant to their journey within the bone marrow (multipotent progenitors of origin, contacts with extracellular matrix molecules, cell-cell interaction, cellular mimicry, etc). The expression of a specific set of surface receptors could be a key determinant for where such EPCs manage to, or fail to, anchor to injured sites of the arterial tree. Hence, some

progenitors might be uniquely designed for the repair of large arteries of the systemic circulation, whereas others are predetermined to anchor to pulmonary arteries. Local challenges to successful landing of EPCs, resulting, for example, from the combined effect of factors such as hemodynamic stresses and expression of adhesive receptors (selectins, integrins, etc), could explain, at least in part, the predilection of arterial branching points and short curvatures for early atherosclerotic lesions.<sup>46,47</sup> The loss of selective groupings of EPCs may explain the uneven distribution of atherosclerotic lesions in the presence of selected risk factors. Thus, it is known that some individuals will display only peripheral arterial lesions, whereas for others, lesion development will have an exquisite selectivity for coronaries.

In summary, both the susceptibility of blood vessels to injury and their amenability to repair and/or remodel are profoundly affected by vascular progenitor cell heterogeneity. Vascular cell morphology can differ dramatically from one vascular bed to another, according to specific functional requirements. Moreover, endothelial cells destined to reside within the arterial or venous vascular beds follow diverging differentiation pathways (Figure 3), and those assigned to these specific locations express characteristic molecular markers. Vessel size, anatomic location, and geometry also determine the quality and magnitude of hemodynamic forces (primary versus secondary currents, flow shear, transmural pressure, etc) to which vascular cells are exposed. Finally, vascular cells are subject to regional molecular cues and signals derived from specialized surrounding tissues. As a result, vascular cell gene expression profiles and functional responses vary significantly from one setting to another<sup>45</sup> and profoundly influence the remodeling and repair capabilities of the cell.<sup>48–50</sup> These topics represent exciting future areas of investigation and may have a profound impact on our understanding of vascular disease and repair.

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