Rapid Endothelial Turnover in Atherosclerosis-Prone Areas Coincides With Stem Cell Repair in Apolipoprotein E–Deficient Mice

Georgios Foteinos, PhD*; Yanhua Hu, MD*; Qingzhong Xiao, PhD; Bernhard Metzler, MD; Qingbo Xu, MD, PhD

Background—Recently, it has been shown that stem/progenitor cells may repair damaged/lost endothelial cells in vein grafts and wire-injured arteries. In the present study, we investigated endothelial cell turnover and regeneration in apolipoprotein E (apoE)−/− transgenic mice carrying LacZ genes driven by an endothelial TIE2 promoter.

Methods and Results—To assess cell proliferation on the surface of aortas in apoE−/− mice and wild-type controls, BrdU was injected into the tail vein and labeled on en face preparation. BrdU-positive cells on the aortas were observed occasionally in wild-type mice and frequently at sites prone to lesion development in apoE−/− mice (0.18±0.1% versus 1.12±0.2%; *P<0.001). Endothelial integrity tests demonstrated that the areas with high rate of cell turnover displayed Evans blue leakage, low levels of VE-cadherin expression, and increased cell attachment, as evidenced by Evans blue dye injection, immunostaining, and scanning electron microscopy, respectively. Furthermore, immunostaining for CD34, Sca-1, Flk-1, and CD133 indicated that 3% to 5% of total cells on the aorta were positive in apoE−/− mice. En face double labeling using Ki-67 and progenitor markers revealed that 30% to 50% of progenitor cells expressed Ki-67, indicating a state of proliferation. To clarify the origin of endothelial progenitor cells participating in endothelial repair in apoE−/− mice, a chimeric mouse model was created by bone marrow transplantation between apoE−/− and LacZ+/apoE−/− mice. Ten months after bone marrow transplantation, 3% to 4% of total cells in the lesion-prone areas were β-gal positive in apoE−/− with apoE−/−/TIE2-LacZ bone marrow mice. When cells of aortas from chimeric mice were cultivated on Matrigel-coated plates, a capillary-like structure was found, which showed β-gal/CD31 or β-gal/von Willebrand factor double positivity. By a combined analysis of laser dissection microscopy and nest reverse transcription polymerase chain reaction, it was found that β-gal+ cells were mainly expressing CD31 and CD144.

Conclusions—Our findings provide the first quantitative data on endothelial turnover and repair by progenitor cells that are, at least in part, derived from bone marrow during development of atherosclerosis in apoE−/− mice. (Circulation. 2008;117:1856-1863.)

Key Words: endothelium ■ endothelium-derived factors ■ pathology ■ progenitor cells ■ stem cells

Atherosclerosis is characterized by endothelial dysfunction and inflammatory response in the arterial tree. It is well known that atherosclerosis develops at preferential sites along the vasculature. These regions experience specific hemodynamic conditions characterized by elevated stretch stress and altered shear stress with a cyclic reversal flow direction (referred to as oscillatory flow). High levels of stretch stress are measured in areas of major curvature, such as the aortic arch and in the vicinity of branch points, sites known to be prone to atherogenesis. Previous, it was shown that structural and functional heterogeneities exist in different regions of the endothelium of large and medium-sized arteries, which is associated with the location of the lesions. Recently, a report demonstrated that areas of low shear stress have a high endothelial death rate in human arteries, suggesting that cells need a high turnover rate to maintain vessel homeostasis. In apolipoprotein E (apoE)–deficient mice, a widely used animal model of atherosclerosis, studies have demonstrated that aging endothelial cells are far more sensitive to apoptosis than younger cells. It is...
conceivable that in older people there is a higher turnover of endothelial cells, especially in areas prone to disturbed blood flow, such as the bifurcations. However, no quantitative data on endothelial turnover in the arterial wall in either humans or apoE−/− mice are available.

After endothelial death, cell regeneration occurs in the areas where acute or chronic stimuli uniquely exist. Although mature endothelial cells have the ability to rapidly replicate in vitro up to ~50 passages, little is known about their in vivo proliferation in situ. Recently, accumulating evidence indicates the existence of circulating endothelial progenitor cells (EPCs) that have an ability to repair injured or dead endothelial cells in animal models. For instance, 2 independent groups investigated the effects of the bone marrow–derived progenitor cells on reendothelialization and neointimal formation after arterial injury. We demonstrated that a large proportion of regenerated endothelial cells in vessel grafts are derived from circulating stem cells. There is also a report showing that EPCs may play a role in regenerating damaged endothelial cells in apoE-deficient mice, but the quantitative data for bone marrow cells to regenerate endothelial cells in the animal are still lacking. Our goal in this study was to demonstrate the in vivo endothelial turnover and involvement of bone marrow–derived stem cells in the repair and regeneration of the endothelium in apoE−/− mice. We demonstrated that atherosclerosis-prone areas have a very high endothelial turnover rate, which is regenerated, at least in part, by bone marrow–derived stem cells.

Methods

Methods are described in detail in the online-only Data Supplement.

Animals

ApoE−/− mice were crossed with TIE2-LacZ mice in our laboratory, and heterozygous offspring were mated to produce apoE-deficient mice expressing β-gal in endothelial cells (TIE2-LacZ/apoE−/−).

Tissue Harvesting and Preparation

Blood was obtained from the inferior vena cava for lipid analysis. For histological analysis, in vivo perfusion with 4% phosphate-buffered formaldehyde was performed, as described previously. For immunostaining, aortic tissues were harvested and stored immediately in liquid nitrogen.

Endothelial Proliferation Assays In Vivo

The in situ proliferation kit FLUOS (Roche, 1810470) was used to assess cell proliferation by the detection of BrdU, a nonradioactive analogue of thymidine, incorporated into the cellular DNA of proliferating cells.

En Face Immunofluorescence Staining

En face prepared aortas were fixed in cold acetone for 10 minutes and air dried for another 10 minutes. The procedure used for immunofluorescent staining was similar to that described previously. Briefly, en face vessels were labeled with rabbit anti-von Willebrand factor antibodies and rat monoclonal antibodies against CD31, CD34, Sca-1, c-Kit, Flk-1 (Abcam Ltd, Cambridge, UK), VE-cadherin, β-gal, and Ki-67 (BD Biosciences), and visualized with swine anti-rabbit Ig or rabbit anti-rat Ig conjugated with FITC or Cy3 (Dakopatts).

Bone Marrow Transplantation

The procedure used for creating chimeric mice was similar to that described previously. Six- to 8-week-old mice received a lethal dose of whole-body x-ray irradiation (950 rad) as described previously. The irradiated recipients received 1 × 107 bone marrow cells. The efficiency of bone marrow transplantation was monitored by β-gal staining and Y-chromosome in situ hybridization for bone marrow sections of chimeric mice. Two types of chimeric mice were created, ie, apoE−/− mice with TIE2-LacZ/apoE−/− bone marrow and TIE2-LacZ/apoE−/− mice with apoE−/− marrow.

X-Gal Staining

The procedure for en face preparation is similar to that described elsewhere. In short, vessel segments were incubated at 37°C for 18 hours in PBS supplemented with 1 mg/mL X-gal.

Endothelial Cell Culture From Mouse Aorta

Aortas from wild-type and apoE−/−/TIE2-LacZ chimeric mice were excised and cleaned free from periadventitial fat and connective tissue, opened longitudinally, and placed, with lumen side down, onto collagen matrix–coated 12-well plates. As a control, smooth muscle cells from mouse aortas were prepared as described previously.

Palm Laser Microdissection and Pressure Catapulting

The cultured cells on the slide were developed with X-gal substrate. Each slide was placed onto the stage of the PALM microlaser. The catapulted cells were then transferred from the lid to the bottom of the microfuge tube by brief centrifugation.

Nest Reverse Transcription Polymerase Chain Reaction

Total RNA was extracted from the cells dissected from Matrigel-coated cultures with the use of the Mini-RNA Isolation Kit according to the manufacturer’s instructions. Oligonucleotide primer sequences for each gene examined in the experiment are shown in the Table.

### Table. Primer Sequences Used in Reverse Transcription Polymerase Chain Reaction

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<tr>
<th>Genes</th>
<th>Primers 5′-3′</th>
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<tr>
<td>CD31</td>
<td>First: sense: acc tgc aaa agc agg tct cag; Antisense: ccg tct ctt cct tct gta gcg</td>
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<tr>
<td></td>
<td>Second: sense: cca cga tgg agt agg tgg gg; Antisense: ccc ctc tga tgg gtt ctc ac</td>
</tr>
<tr>
<td>CD144</td>
<td>First: sense: cta ctc tct gca gaa agg gc; Antisense: ctc aga ccc tgt tgg tgg tgc</td>
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<tr>
<td></td>
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<tr>
<td>SM22</td>
<td>First: sense: gca gtc caa aat tga gaa ga; Antisense: ctc tgt tgg tct ctc cgg tct gaa gaa</td>
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increased number of BrdU-positive cells in the aortas of apoE−/− mice. Three hours after BrdU injection, aortas were harvested, en face prepared, and labeled for BrdU. Panels a (×100) and b (×400) are from wild-type mice with few positive cells. Panels c (×100) and d (×400) are from apoE−/− mice with many proliferating cells around a branch in the vessel of aorta. The graphic data are mean±SEM of BrdU-positive cells per aorta (e) and percentage of BrdU-positive cells (f). *Significant difference between 2 groups, P<0.001 (n=6).

Statistical Analysis
Statistical analyses were performed with the Student t test. Results are given as mean±SEM. A value of P<0.05 was considered significant.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results
Endothelial Turnover on Aortas of ApoE−/− Mice
To assess cell proliferation on the surface of aortas in apoE−/− mice and wild-type controls, BrdU was injected into the tail vein, and specific staining for BrdU was employed as described in detail in Methods. As shown in Figure 1, BrdU-positive cells on en face labeling on the surface of aortas were observed occasionally in wild-type mice (Figure 1a and 1b) and frequently at sites prone to lesion development, such as bifurcations and sites of increased blood flow in apoE−/− mice (Figure 1c and 1d). The number of BrdU-positive cells appeared greater in apoE−/− mice than in the wild-type controls. Under high magnification, positive staining was mainly located in the nuclear area near the lesion-prone areas in apoE−/− aortas (Figure 1d). Semiquantitative data shown in the graphic indicates that there were an increased number of BrdU-positive cells in the aortas of apoE−/− mice compared with wild-type controls (Figure 1e and 1f). There was an 8-fold difference in BrdU-positive cells between apoE−/− and wild-type mice, indicating a higher proliferating rate of aortic cells in apoE−/− animals.

To test the hypothesis that endothelial areas with proliferating neocells may lack endothelial function, 3 experiments, that is, Evans blue leakage, VE-cadherin staining, and electron microscopy, were conducted in wild-type and apoE−/− mice. For the Evans blue test, 3 groups of mice with 4 animals in each group were used, that is, wild-type and younger (12 weeks) and older (32 weeks) apoE−/− mice. Data shown in Figure 2a to 2c demonstrate that the extent of the Evans blue staining on the surface of the aortas was different between wild-type and apoE−/− mice. Figure 2c shows a great degree of positive blue staining around the opening of the branch in older apoE−/− mice under higher magnification. In contrast, the extent of the blue staining was much less in the younger apoE−/− mice (Figure 2b) and virtually absent in the wild-type controls (Figure 2a). No difference in Evan’s blue staining between 12- and 32-week-old wild-type mice was found (data not shown). The same trend of staining was followed when blue-stained areas on the aortas were quantified on their full length in Figure 2g, indicating that endothelial leakage occurs in the aortic wall of apoE−/− mice. Because VE-cadherin is a key protein composed of tight junctions between endothelial cells, we demonstrated that this protein was altered in apoE−/− mice, as indicated in Figure 2d, 2e, and 2f. This result further supports the notion of endothelial dysfunction in apoE−/− mice.

To verify the results of endothelial dysfunction and visualize the changes of endothelial morphology, we used scanning electron microscopy. The aortas of the wild-type mice had an intact and smooth endothelium (Figure 3a), whereas apoE−/− mice (Figure 3b to 3e) displayed endothelial changes with cells peeling off the surface of the intima. Higher magnification in Figure 3d and 3e indicates that some cells attached to the surface or penetrated between endothelial cells, as indicated by the arrow. Thus, characteristics of arteries in apoE−/− mice are morphological alterations and dysfunction in the areas with proliferating (neo)endothelial cells.

Figure 1. En face photographs showing Brdu-positive cells of mouse aortas. Three hours after BrdU injection, aortas were harvested, en face prepared, and labeled for BrdU. Panels a (×100) and b (×400) are from wild-type mice with few positive cells. Panels c (×100) and d (×400) are from apoE−/− mice with many proliferating cells around a branch in the vessel of aorta. The graphic data are mean±SEM of Brdu-positive cells per aorta (e) and percentage of Brdu-positive cells (f). *Significant difference between 2 groups, P<0.001 (n=6).

Figure 2. En face photographs showing Evans blue leakage test and immunostaining for VE-cadherin. After Evans blue dye injection into tail vein of apoE−/− (a) and apoE+/− (b, aged 12 weeks and c, aged 32 weeks) mice, aortas were harvested and washed. Blue-stained areas were photographed (a to c) and quantified (g). For immunostaining, aortas from apoE−/− (d) and apoE+/− (e and f) mice were harvested, en face prepared, fixed with acetone, and labeled with anti-VE-cadherin antibody and counterstained with DAPI for nuclear visualization. Magnification ×50 (a to c); ×400 (d to f). Panel g is statistical data of 4 animals each group. *Significant difference from other groups, P<0.01.
Progenitor Cell Repair for Endothelium

The BrdU incorporation study in the previous experiment revealed extensive proliferation of cells on the aortas of apoE−/− mice. The question arose of whether these cells were neighboring endothelial cells that underwent proliferation or whether they were EPCs that were recruited to replace the damaged endothelium at the sites of injury and mature to endothelial cells. Immunostaining for progenitor markers, eg, CD34+, Sca-1+, c-Kit+, and Flk-1+, was used to identify and compare populations of cells on the aortas of apoE−/− as well as wild-type mice. As expected, CD31-positive staining was observed in almost all cells located on the surface of aortas (Figure 4b), whereas the negative control using rat Ig as a primary antibody showed no staining for the cells (Figure 4a). As Figure 4c to 4f shows, CD34- and Sca-1–, c-Kit–, and Flk-1–positive cells were the most abundant, whereas there were few if any CD133+ cells (data not shown). Figure 4g shows quantitative data of positively stained cells and indicates that 3% to 5% of total cells on the surface of the vessel were progenitor marker positive. In addition, very few cells (<0.003%) displayed positivity with marker CD34 and Sca-1, c-Kit, or Flk-1 in the aortas of wild-type mice.

To answer the question of whether progenitor cell marker–positive cells are proliferating, we used double immunofluorescence staining on the aortas using Ki-67 as the proliferation marker plus the aforementioned markers. There were a significant number of Sca-1/Ki-67 double positive cells on the aortas of apoE−/− mice, as shown in Figure 5d to 5f, whereas no staining in negative controls was seen (Figure 5a to 5c). A similar pattern was found for CD34/Ki-67 and Flk-1/Ki-67 double staining, ie, 30% to 50% of EPC marker–positive cells showed Ki-67

Figure 3. Scanning electron microscope photographs. Aortas from apoE−/− (a) and apoE−/− (b to e) mice were harvested and prepared for scanning electron microscopy as described in Methods. The arrow indicates an attached cell between endothelial cells. Magnification ×50 (a and b); ×100 (c); ×4000 (d and e).

Figure 4. En face immunostaining for progenitor markers on aortas. ApoE−/− mice aged 32 weeks were fed with normal chow diet. Aortas were en face prepared and labeled with different rat monoclonal antibodies and counterstained with DAPI for nuclear visualization. Panel a serves as a negative control using normal rat Ig. Magnification ×400. Ctl− indicates negative control. Graphic data in panel g are mean ± SEM (n=6).

Figure 5. En face double immunostaining for progenitor and proliferating markers on aortas. ApoE−/− mice aged 32 weeks were fed with normal chow diet. Aortas were en face prepared and labeled with rat monoclonal antibodies. Panels a to c serve as negative controls using normal rat Ig instead of rat monoclonal antibodies. Ctl− indicates negative control. Magnification ×400.
apoE mice that received apoE−/−/TIE2-LacZ bone marrow were maintained with normal chow diet for 10 months. Aortas from apoE−/− (a), apoE−/−/TIE2-LacZ (b), or chimeric (c to e; apoE−/− mice that received apoE−/−/TIE2-LacZ bone marrow) were en face prepared and developed with X-gal. Blue color indicates β-gal+ cells (%).

Figure 6. En face staining for β-gal on chimeric aortas. ApoE−/− mice that received apoE−/−/TIE2-LacZ bone marrow were maintained with normal chow diet for 10 months. Aortas from apoE−/− (a), apoE−/−/TIE2-LacZ (b), or chimeric (c to e; apoE−/− mice that received apoE−/−/TIE2-LacZ bone marrow) were en face prepared and developed with X-gal. Blue color indicates positivity, indicating that a proportion of progenitor cell marker–positive cells are proliferating. Because currently there is no conclusive marker for EPCs, it is believed that the cells could be EPCs if they are double positive for both markers used in the present study. Double staining demonstrated that the majority of Sca-1+ cells were c-Kit positive or Flk-1 and CD34 double positive in the aortas of apoE−/− mice (Figure 5g to 5l), indicating that a proportion of proliferating cells of the endothelium are derived from EPCs.

**Contribution of Bone Marrow Stem Cells**

To further clarify the origin of stem/progenitor cells participating in endothelial repair in apoE−/− mice, a chimeric mouse model was created by bone marrow transplantation between apoE−/− and LacZ+/apoE−/− mice. Before bone marrow transplantation, endothelial cells of apoE−/− mice as a recipient showed a negative staining for β-gal (Figure 6a), whereas bone marrow donors of LacZ+/apoE−/− mice displayed blue staining on the endothelial layer (Figure 6b). Ten months after bone marrow transplantation, a number of LacZ+ cells on the surface of aortas from apoE−/− with TIE2-LacZ bone marrow mice were observed, especially around the branching areas, where atherosclerotic lesions were located (Figure 6c). With higher magnification, a single β-gal+ cell was occasionally seen to cover a foam cell (Figure 6d), indicating that bone marrow–derived cells participate in lesion development in the early stage. At the late stage, abundant β-gal+ cells were localized around the transition zone between lesions and normal intimal areas (Figure 6e). The graphic data shown in Figure 6f are summarized results from 8 chimeric animals. Approximately 3% to 4% of total endothelial cells in the lesion-prone areas were β-gal positive, and there were relatively few in lesion-resistant areas, suggesting that bone marrow–derived cells may repair damaged endothelial cells in apoE−/− mice.

To further characterize bone marrow–derived cells, aortic endothelial cells from chimeric mice, that is, apoE−/− with TIE2-LacZ/apoE−/− bone marrow for 10 months, were cultured on collagen IV– (Figure 7a and 7b) and Matrigel-coated (Figure 7c to 7f) chamber slide bottles, respectively. Figure 7a, 7b, and 7c shows data of cultured cells developed with X-gal, indicating that some cells were β-gal positive. When cells were cultivated on Matrigel-coated bottles, a capillary-like structure was found (Figure 7c to 7f). Figure 7d, 7e, and 7f shows negative controls with the use of normal rat Ig. Double immunofluorescence staining revealed that β-gal+ cells were CD31 and von Willebrand factor positive (Figure 7g to 7l). Furthermore, β-gal+ cells (≈100 cells per sample) from Matrigel-coated cultures were collected with a laser dissection microscope, and a nest reverse transcription polymerase chain reaction was performed. As shown in Figure 7m, β-gal+ cells (S1 and S2) expressed both CD31 and CD144 endothelial markers. Interestingly, these cells also expressed Flk-1 and lightly expressed Sca-1 (Figure 7m), suggesting a progenitor cell origin.
Endothelial Cell Turnover in Lesion-Prone Areas

ApoE−/− mice have been widely used by many investigators, as evidenced by many publications (>30 000 on PubMed). These animals display many characteristics of human spontaneous atherosclerosis, eg, lesion locations at sites of disturbed blood flow, such as in the spinal and iliac bifurcation and aortic arch.23 Second, the endothelial layer present at these sites is under constant stress, which leads eventually to stress-induced apoptosis.24 We thought that mapping endothelial cell turnover on whole aortas would provide basic information. To investigate this issue, the numbers of proliferating cells on the aortas of apoE−/− mice as well as wild-type animals were evaluated by BrdU immunostaining. The results confirmed our hypothesis, ie, there was a significant difference in the number of proliferating cells on the aortas of apoE−/− and wild-type mice. Importantly, lesion-prone areas display a higher turnover rate of endothelial cells, as indicated by BrdU-positive staining. This is a clear attempt of the organism to regenerate its injured endothelium and compensate for the cell loss. The need for endothelial cell healing and regeneration is much greater in apoE−/− mice because hypercholesterolemia has developed over time, and the cells at these sites are under great stress. Therefore, endothelial cells in these areas undergo death/proliferation because of hyperlipidemia and mechanical stress–induced unfavorable conditions.

Endothelial Dysfunction and Lesion Development

It has been known that endothelial dysfunction is a key event in the pathogenesis of atherosclerosis.25 We provide direct and systemic evidence showing endothelial dysfunction where atherosclerotic lesions are localized. This was demonstrated by the Evans blue injection test, which clearly illustrated that increased endothelial leakage is evident around the sites of bifurcations in apoE−/− mice and that this leakage is significantly low in wild-type mice. Similarly, the whole aorta of apoE−/− mice has more significantly intense staining compared with controls. Whereas the aortas of the control animals have a smooth and intact endothelium, it is clear that the aortic endothelium of apoE−/− animals is extensively damaged, with cells peeling off the surface of the intima, as evidenced by scanning electron microscopy. Furthermore, the tight junction of endothelial cells in apoE−/− mice seems markedly lost. It was also observed that lesion development is strictly correlated with the degree of endothelial leakage and VE-cadherin expression (data not shown). Taken together, these data establish the relationship between the cell turnover rate, endothelial dysfunction, and atherosclerosis development in apoE−/− mice.

Discussion

It is well known that atherosclerotic lesions in the arteries are localized in some areas where blood flow is disturbed, resulting in endothelial dysfunction in the presence of hyperlipidemia.2 The alterations of endothelial cells in these areas could be crucial in determining lesion development, but less is known about endothelial death and regeneration. In the present study, we provided quantitative data on endothelial turnover in whole aorta and mapped the location of high-turnover areas of endothelial cells, which are colocalized with endothelial dysfunction, for example, Evans blue dye leakage and decreased VE-cadherin expression, and lesion location. Importantly, we demonstrated that stem cells contribute to the repair of severely damaged endothelial cells in high-turnover areas, although they have not been shown to exhibit the full function of mature endothelial cells. Thus, our findings could be crucial for understanding the cellular mechanisms of atherosclerosis development, offering basic information for therapeutic intervention in the future.
Stem Cells Contribute to Endothelial Repair

After endothelial death, the endothelium must be repaired by either neighboring endothelial replication or stem cells in the blood or the vessel wall.11 Although there is no doubt that neighboring endothelial cells play an essential role in repairing damaged cells, recent findings also suggest the potential role of EPCs in endogenous regeneration, especially in extensive loss of the cells.9,12,13 Studies from our group have provided crucial evidence of the involvement of stem/progenitor cells in the regeneration of the vascular endothelium.14 Xu et al.26 provided the first evidence that EPCs, mainly from the circulation and partially from the bone marrow, are responsible for repairing the injured vessels in murine vein grafts. In the present study, we further demonstrated the involvement of EPCs in the repair and regeneration of the damaged endothelium in spontaneous hyperlipidemia-induced atherosclerosis in vivo. A panel of stem/progenitor cell markers, e.g., CD34, Sca-1, Flk-1, and CD133, was tested because no conclusive single marker is available for EPC recognition. We demonstrated that ∼3% to 5% of cells on the surface are progenitor marker positive in apoE−/− mice, which could be significant because a proportion of EPCs will lose their marker when they differentiate into mature endothelial cells. Interestingly, data from double immunofluorescence staining with progenitor cell markers and the proliferation marker Ki-67 indicate that a significant number of the cells at these sites are indeed proliferating in situ. This presents strong in vivo evidence that stem/progenitor cells are a key part of endothelial repair and maintenance of vessel wall integrity.

Bone marrow as an EPC source has been recognized and may play a role in angiogenesis, although controversial results concerning the physical participation of EPCs in vessel formation exist.27–33 In terms of large-vessel repair, reports from several groups demonstrate the incorporation of EPCs into endothelium after severe damage/injury of vessels in animal models.12,13,26,34 In the present study, we provide quantitative data showing that bone marrow–derived stem cells are a source of a proportion of endothelial cells present in the areas where the endothelial turnover rate is high. Convincingly, cultured cells from the surface of aortas express both β-gal and endothelial markers and form a capillary-like structure in Matrigel culture that expresses markers of both endothelial and progenitor cells (Figure 7). In wild-type mice, no bone marrow–derived cells on the endothelium of aortas were observed 10 months after chimeric establishment (data not shown), which could serve as a “negative” control. The percentage of bone marrow–derived cells is ∼1% to 2% of total endothelial cells, but it is higher in lesion-prone areas. Our data establish the contribution of bone marrow cells to endothelial repair in apoE−/− mice. On the other hand, accumulating data indicate that non–bone marrow stem/progenitor cells contribute to repair damaged endothelial cells in several animal models.9,11,35 Thus, endothelial regeneration in apoE−/− mice by stem cells should be higher if non–bone marrow–derived stem cells are taken into account.

The fact that we demonstrate that higher numbers of progenitor cells in apoE−/− mice are correlated with increased atherosclerosis has several implications. First, as atherosclerosis develops, endothelial turnover is high, and the demand for endothelial progenitors is greater. EPCs in circulation become exhausted. Supporting this notion is the fact that the number of EPCs in blood and bone marrow of old apoE-deficient mice is significantly lower than that of wild-type mice.15 The decreased number of progenitor cells in patients with atherosclerosis was reported by several groups.36–38 This implication is also supported by a mathematical model of endothelial layer maintenance showing that the age of the endothelium depends on the homing rates of EPCs.39 Second, we suggest that EPCs may not have an endothelial function at the early stage when they home to the surface of the vessel, resulting in endothelial leakage/dysfunction. Finally, EPCs may have the ability to differentiate into other types of cells, e.g., smooth muscle cells, which might be incorporated into the lesion and promote lesion development. Evidence to support this hypothesis includes the fact that treatment with the anti-CD34 antibody–coated stent used in the injured vessel results in promotion of reendothelialization but also enhancement of neointimal lesions.40 Therefore, we hypothesize that EPCs could be a double-edged sword for vascular integrity and lesion development. If more detailed mechanisms of EPC homing and differentiation can be obtained, we may use a specific inhibitor/drug to control their behavior and function toward wound healing without a scar or lesion.

In summary, the risk factors for atherosclerosis result in endothelial high turnover, coinciding with the loss of functionality and gradual depletion of progenitors, leading to insufficient and improper endothelial healing that might result in increasing the atherosclerotic burden. At the time of both endothelial death and stem/progenitor cell homing, the integrity of the endothelium is disrupted, causing endothelial leakage/dysfunction. We therefore demonstrate the involvement of stem/progenitor cells in atherosclerosis as a repair mechanism and suggest that understanding their role in repair of the vessel may be as important as understanding their role in the development of atherosclerosis.

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Disclosures

None.

References

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