Circulating Endothelial Progenitor Cells Do Not Contribute to Plaque Endothelium in Murine Atherosclerosis

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Background—It has been reported that circulating endothelial progenitor cells (EPCs) home to and differentiate into endothelial cells after various kinds of arterial injury. By inference, EPCs are also proposed to be important in the most important arterial disease, atherosclerosis, but the evidence for this theory is not clear. In the present study, we assessed the contribution of circulating EPCs to plaque endothelium in apolipoprotein E–deficient (apoE−/−) mice.

Methods and Results—To investigate whether EPCs in the circulating blood are a source of plaque endothelial cells during atherogenesis, we examined plaques in lethally irradiated apoE−/− mice reconstituted with bone marrow cells from enhanced green fluorescent protein (eGFP) transgenic apoE−/− mice and plaques induced in segments of common carotid artery transplanted from apoE−/− mice into eGFP+apoE−/− mice. Among 4232 endothelial cells identified by a cell-type–specific marker (von Willebrand factor) and analyzed by high-resolution microscopy, we found only 1 eGFP+. Using the Y chromosome to track cells after sex-mismatched transplants yielded similar results. To investigate whether circulating EPCs are involved in plaque reendothelialization after plaque disruption and superimposed thrombosis, we produced mechanical plaque disruptions in carotid bifurcation plaques in old lethally irradiated apoE−/− mice reconstituted with eGFP+apoE−/− bone marrow cells and carotid bifurcation plaques transplanted from old apoE−/− mice into eGFP+apoE−/− mice. Only 1 eGFP+ endothelial cell was found among 3170 analyzed.

Conclusions—Circulating EPCs rarely, if ever, contribute to plaque endothelium in apoE−/− mice. These findings bring into question the prevailing theory that circulating EPCs play an important role in atherogenesis. (Circulation. 2010;121:898-905.)

Key Words: atherosclerosis ■ endothelial progenitor cells ■ endothelium ■ thrombosis ■ von Willebrand factor

In a seminal study in 1997, Asahara et al1 isolated putative endothelial progenitor cells (EPCs) from the peripheral blood. During short-term culturing, some of these cells acquired endothelium-like characteristics, and they homed to sites of angiogenesis when injected intravenously into animals with hind-limb ischemia. Subsequently, endogenous circulating EPCs were reported to home to sites of neovascularization, differentiate into endothelial cells (ECs) in situ,2,3 and participate in endothelial regeneration after various kinds of arterial injuries, including mechanical injury,4–6 transplant arteriopathy,4,7 and vein graft atherosclerosis.8 The bone marrow (BM) is considered to be the major but not the only source of circulating EPCs.9,10

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Although atherosclerosis is by far the most important arterial disease worldwide, little is known about putative EPCs in this disease. Only indirect evidence exists for the prevailing understanding that circulating EPCs provide protection against atherosclerosis by their innate ability to replace dysfunctional ECs and to regenerate senescent and damaged endothelium.11,12

The term EPC was initially used in the literally correct way for immature precursor cells capable of differentiating into mature ECs in vivo1–8; however, some have gradually redefined the term to include circulating “angiogenic” cells without a putative endothelial fate because it was discovered that many cells fulfilling the original criteria used to define EPCs promote angiogenesis and endothelial proliferation solely by paracrine activities without belonging to the endothelial lineage (reviewed elsewhere10,13). To avoid confusion, we adhere to the literally correct definition of a progenitor cell as an immature precursor cell capable of differentiating into a mature cell type in vivo.

In the present study, we determined the origin of plaque ECs during atherogenesis and after plaque disruption in the most commonly used animal model of atherosclerosis: the apolipoprotein E–deficient (apoE−/−) mouse. We found few, if any, BM-derived or other bloodborne ECs over atheroscle-
EC origin in atherosclerosis

Experiment 1: Tracking of hematopoietic stem cell progeny

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Figure 1. Overview of experiments. A, Origin of ECs in atherosclerosis using eGFP or Y chromosome as tracking markers in eGFP+ apoE-/- BM→apoE-/- transplanted mice (experiment 1) and in transplanted CCA segments between apoE-/- and eGFP+ apoE->/- mice (experiment 2). B, EC origin after mechanical plaque disruption (PD) of CB plaques using eGFP as tracking marker in eGFP+ apoE->/- BM→apoE-/- transplanted mice (experiment 3) and in CB plaques transplanted between apoE-/- and eGFP+ apoE->/- mice (experiment 4). BMT indicates BM transplantation; CCA, common carotid artery; CB, carotid bifurcation; HF, high-fat diet; CCAT, CCA segment transplantation; CBT, CB transplantation.

Methods

See the online-only Data Supplement for an expanded Methods section.

Transgenic Animals

Apoe-/- mice (Taconic M&B, Ry, Denmark), backcrossed >10 times to C57BL/6 mice, and enhanced green fluorescent protein-positive (eGFP+) C57BL/6 mice (Jackson Laboratories, Bar Harbor, Me) were intercrossed to obtain eGFP+ apoE-/- mice. Schematics of the experimental design are shown in Figures 1 and 2. The mice analyzed in experiments 1, 3, and 4 originated from previously published experiments on the origin of atherosclerotic plaque smooth muscle cells. All of the procedures involving experimental animals were approved by the Danish Animal Experiments Inspectorat.

Experiment 1: Tracking Hematopoietic Stem Cell Progeny in Atherosclerosis

To investigate whether hematopoietic stem cells contribute to plaque ECs during atherogenesis, we examined plaques (in the aortic root, aortic arch, brachiocephalic trunk, and abdominal aorta) from eGFP+ apoE-/- BM→apoE-/- transplanted mice. Briefly, apoE-/- mice (n=17; male or female; age, 8 weeks) were lethally irradiated and rescued by a tail-vein injection of 10⁷ sex-mismatched BM cells from eGFP+ apoE-/- mice (Figures 1A and 2). Hematopoietic chimerism was determined by flow cytometry. Age-matched apoE-/- mice (n=5) and eGFP+ apoE-/- mice (n=6) were included as controls. Mice were fed a high-fat diet (21% saturated fat, 0.25% cholesterol; Hope Farms, Woerden, the Netherlands) to accelerate atherogenesis and euthanized at 20 or 32 weeks of age.

Experiment 2: Tracking Blood-Derived Cells in Collar-Induced Atherosclerosis

To investigate whether any types of bloodborne cells contribute to plaque ECs during atherogenesis, we performed orthotopic transplantations of common carotid artery (CCA) segments from apoE-/- into eGFP+ apoE-/- mice (n=9) or from eGFP+ apoE-/- mice (n=5) (isotransplantation except for the eGFP transgene) (Figures 1A and 2). Four mice had a sex-mismatched (female-to-male) CCA transplant. The others were sex-matched (male-female). The transplantation technique was essentially as described previously, except that 5 sutures were used for each end-to-end anastomosis. After 4 weeks, mice were put on a high-fat diet. Two weeks later, localized atherosclerosis was induced by placement of a constrictive perivascular collar around the distal part of the CCA segment as described. All of the mice were euthanized 10 weeks after collar placement. Plaques developed proximal to the constrictive collar with an area of plaque-free arterial wall separating the lesion from the proximal atherosclerosis site.

Experiment 3: Tracking Hematopoietic Stem Cell Progeny After Mechanical Plaque Disruption

To investigate whether EPCs of hematopoietic origin contribute to endothelial regeneration after plaque disruption, we examined atherosclerotic plaques 1 or 4 weeks after mechanical plaque disruption had been produced by endovascular needle injury (Figures 1B and 2). Briefly, old apoE-/- mice (n=15; male; age, 18 months) were BM transplanted with 10⁷ donor BM cells from eGFP+ apoE-/- mice.
(male; age, 2 to 3 months). Age-matched apoE⁻/⁻ (n=6) and eGFP⁺apoE⁻/⁻ (n=4) mice were included as controls. At 4 weeks after transplantation, hematopoietic chimera was tested by flow cytometry of peripheral leukocytes. At 6 weeks after transplantation, carotid bifurcation (CB) plaques were isolated, and the CCA and carotid branches were temporarily ligated. A 9-0 suture was then inserted into the lumen of the CCA, advanced through the artery, and repeatedly inserted into the plaque, leading to consistent plaque damage with superimposed thrombosis as previously described.15 BM-transplanted mice were euthanized 1 or 4 weeks after mechanical plaque disruption. Controls were euthanized after 4 weeks.

**Experiment 4: Tracking Blood-Derived Cells After Mechanical Plaque Disruption**

To investigate whether any types of circulating cells contribute to endothelial regeneration after plaque disruption, we analyzed healed CB plaques that were transplanted from old apoE⁻/⁻ mice (age, 12 to 17 months) into young eGFP⁺apoE⁻/⁻ mice (age, 12 weeks; n=9) or from old eGFP⁺apoE⁻/⁻ mice (15 months) into young apoE⁻/⁻ mice (age, 12 weeks; n=5) before mechanical plaque disruption (Figures 1B and 2).15 Briefly, CBs with advanced atherosclerotic plaques were inserted into the CCA of recipients using either the external or internal carotid artery for the distal anastomosis depending on the location of the plaque. The carotid branch not used for anastomosis was ligated. Immediately after transplantation, mechanical disruption of the CB plaque was produced as described above. Mice were euthanized after 4 weeks.

**Tissue Processing**

Anesthetized mice (pentobarbital 5 mg IP) were killed by exsanguination, perfusion fixed with phosphate-buffered 4% formaldehyde (pH 7.2) through the left ventricle for 5 minutes, and immersion fixed for 6 hours at room temperature. Tissues were cryoprotected in sucrose solution (25% wt/vol for 24 hours plus 50% wt/vol for 24 hours), embedded in OCT compound (Sakura Finetek, Zoeterwoude, the Netherlands), and snap-frozen in liquid nitrogen–chilled methanol:acetone (1:1). Specimens were cut into 4-μm-thick sections.

**Immunohistochemistry**

ECs were identified by staining for von Willebrand Factor (vWF) (polyclonal rabbit antihuman vWF; A0082; Dako, Glostrup, Denmark; 1:200 dilution) for 1 hour, after blocking of unspecific binding with normal goat serum (Jackson ImmunoResearch, West Grove, Pa; 10%) for 30 minutes, followed by staining for 1 hour with Texas Red–conjugated goat anti-rabbit secondary antibody (Jackson ImmunoResearch; 1:200 dilution). IgG antibodies from nonimmunized rabbit at the same concentration served as a negative control (rabbit IgG, X0903, Dako). All of the antibodies were diluted in 1% normal goat serum in TBS. Cell nuclei were stained with DAPI (Sigma, St Louis, Mo). Sections were mounted in Slowfade Light Antifade (Invitrogen, Carlsbad, Calif). eGFP was detected by its natural fluorescence.

**Fluorescence In Situ Hybridization**

Detection and localization of Y chromosomes were done in a subset of vWF-stained sections from mice that received a sex-mismatched BM or CCA transplant in experiments 1 and 2. The procedure was done as previously described.14 First, images of vWF-stained sections were acquired and stored. Second, the coverslip was removed and tissue sections were treated in 10 mmol/L sodium citrate, followed by digestion in 0.05% pepsin solution. The Y chromosome was then detected by a FITC-conjugated paint probe (Cambio, Cambridge, England), counterstained with DAPI solution, and mounted in Slowfade Light Antifade (Invitrogen).

**Microscopic Analysis**

Serial sections of plaques were analyzed in an Olympus Cell-R epifluorescence microscope system followed by software deconvolution. Briefly, wide-field z-axis image stacks (31 layers spanning 10 μm) were acquired using the motorized focus and deconvoluted using a blind 3-dimensional deconvolution algorithm (Autoquant Deblur 9.3, Autoquant Imaging, Troy, NY). A single optical section through the center of the section was examined. Only ECs, defined as nucleated cells with clear intracellular location of vWF, were analyzed for colocalization of eGFP signal. For fluorescence in situ hybridization (FISH) analysis, the deconvoluted optical sections were combined into a single image (the maximum projection) to visualize all Y chromosomes in the specimen. Differential interference contrast microscopy was used to resolve cellular borders and morphology.

**Results**

The plaque endothelium was identified as a layer of thin, elongated vWF⁺ cells bordering the lumen. Staining of vWF was localized to the cytoplasm in a granular pattern corresponding to its storage in Weibel-Palade bodies. vWF was found to be a sensitive marker for ECs on the plaque surface; only very few cells that by morphology were suspected to be plaque ECs were vWF negative. As expected and indicating leaky endothelium, variable, extracellular staining of matrix-bound vWF was seen in plaques in addition to intracellular endothelial staining. No microvascular vWF⁺ ECs were detected in the plaque interior. No staining was observed with the isotype control antibody.

**Origin of ECs During Atherogenesis**

**No Atherosclerotic Plaque ECs of Hematopoietic Origin**

To test the hypothesis that circulating EPCs of hematopoietic origin contribute to plaque ECs, we examined atherosclerotic plaques developing in eGFP⁺apoE⁻/⁻ BM–apoE⁻/⁻ transplanted mice (experiment 1, Figure 1A and 2). Long-term reconstitution of the hematopoietic system with eGFP⁺ cells was confirmed by flow cytometry of peripheral blood leukocytes (see Table I of the online-only Data Supplement). Lesions were mostly of the fibrofatty type. With 1 exception, we found no eGFP⁺vWF⁺ cells (indicating hematopoietic origin) among 2539 vWF⁺ cell profiles analyzed in 17 mice (Figure 3a through 3d). One EC appeared to coexpress eGFP. Closer examination with differential interference contrast microscopy to resolve cellular borders, however, could not exclude the possibility that what appeared to be a double-positive cell could be an eGFP⁺ cell infiltrating an eGFP⁻vWF⁺ endothelium (see Figure I of the online-only Data Supplement).

In positive control eGFP⁺apoE⁻/⁻ mice (n=6), the majority of (437 of 738 [59%]) but not all, vWF⁺ ECs were eGFP⁺, whereas no eGFP⁺ cells were detected in negative control apoE⁻/⁻ mice (578 vWF⁺ cells analyzed; n=5). The incomplete eGFP expression in ECs of eGFP⁺apoE⁻/⁻ mice has, to the best of our knowledge, not been reported before. It was not caused by deterioration of the eGFP signal during storage of our specimens because incomplete eGFP expression was also seen in freshly harvested plaques (424 eGFP⁺vWF⁺ ECs among 552 vWF⁺ cells analyzed [77%] n=6). Lack of eGFP transgene expression in ECs of eGFP⁺apoE⁻/⁻ mice may occur at random, in which case eGFP is a sufficient tracking marker for our studies. Lack of eGFP expression, however, could also be confined to a separate EC subpopulation, and EPCs may in theory contribute selectively to this subset. To rule out this possibility, we used the Y chromosome as an independent tracking marker for hematopoietic origin in plaques from male-
to-female BM-transplanted mice (n=7). Not once did we detect a Y chromosome among 352 vWF+ EC profiles analyzed (Figures 3e and f). In contrast, the Y chromosome was detected in 33 of 82 vWF+ EC profiles (40%) in plaques from female-to-male BM-transplanted mice (n=4). This fraction was expected because of the thickness of the sections and the EC nucleus size.

Figure 3. No ECs of hematopoietic origin in atherosclerotic plaques. a, An aortic root plaque from a male eGFP+apoE−/− BM→female apoE−/− transplanted mouse (32 weeks of age) exhibiting donor-derived eGFP+ foam cells (green) and recipient-derived vWF+ cells (red only). Fluorescence microscopy is combined with DIC imaging (grayscale) to reveal tissue structure. b through d, Higher magnification of the area demarcated in a. No eGFP+vWF+ double-positive cells are present. e and f, Sequential immunostaining and FISH technique revealed no male vWF+ cells. Arrows indicate nuclei that contain a Y chromosome as detected by subsequent FISH of the same section. f, FISH for Y chromosome (green) performed on the same section. Red indicates vWF+; green, eGFP; blue, DAPI; grayscale, DIC; L, lumen; F, foam cells; and C, cholesterol crystals.

Atherosclerotic Plaque ECs Are Derived From the Local Vessel Wall

Experiment 1 showed that EPCs of hematopoietic origin contribute extremely rarely, if at all, to plaque ECs. To examine whether any bloodborne (including nonhematopoietic) cells home and differentiate to plaque ECs during atherogenesis, we analyzed constrictive collar-induced plaques in transplanted CCA segments (experiment 2, Figures 1A and 2). In plaques developing in apoE−/− CCA segments grafted into eGFP+apoE−/− mice (n=9), no eGFP+vWF+ cells were found among 1074 vWF+ cell profiles analyzed in serial sections (Figures 4a through c). In the inverse experiment, in which plaques were induced in eGFP+apoE−/− CCA segments grafted into apoE−/− mice (n=5), 230 of 301 vWF+ cell profiles (76%) were eGFP+ (Figures 4d through f).

Consistently, no Y chromosome–positive vWF+ cells (among 260 vWF+ cells analyzed) were detected in plaques from the subset of mice (n=4) in which the CCA transplantation was performed from female to male (Figure 5). As a positive control, the Y chromosome in 46 of 95 vWF+ cells (48%) were detected in plaques developing in male-to-male CCA transplanted segments (n=4).

Origin of Regenerated ECs After Plaque Disruption

These first experiments demonstrated that plaque ECs lost during atherogenesis are replenished by local cells rather than...
by circulating EPCs, but they did not address what may occur after atherosclerotic plaque rupture with superimposed thrombosis. Plaque ruptures, most of them silent, are common in human atherosclerosis but not in mice, and recent findings suggest that activated platelets adhering to ruptured plaques may facilitate homing of circulating EPCs. To test this hypothesis, we examined CB plaques that had been subjected to mechanical plaque disruption by our recently described needle injury technique. This procedure causes consistent plaque surface injury with loss of plaque endothelium and the formation of a platelet-rich thrombus. Four weeks later, the thrombus was no longer present, and with few exceptions, the healed plaques were covered by an intact layer of ECs, indicating that endothelial regeneration had occurred.

Regenerated ECs After Plaque Disruption Are Not of Hematopoietic Origin

To analyze whether EPCs of hematopoietic origin contribute to endothelial regeneration (experiment 3, Figures 1B and 2), we examined eGFP<sup>+</sup> apoE<sup>−/−</sup> BM→apoE<sup>−/−</sup> transplanted mice at 1 week (n=4) and 4 weeks (n=6) after plaque disruption. Reconstitution of the hematopoietic system with eGFP<sup>+</sup> cells in these mice was confirmed by flow cytometry (see Table I of the online-only Data Supplement). Among 434 vWF<sup>+</sup> cells analyzed after 1 week and 972 vWF<sup>+</sup> cells analyzed after 4 weeks, not a single eGFP<sup>+</sup> vWF<sup>+</sup> double-positive cell was identified (Figure 6). In comparison, 72% of vWF<sup>+</sup> cells (445 of 615 analyzed) were eGFP<sup>+</sup> in healed plaques in positive control eGFP<sup>+</sup> apoE<sup>−/−</sup> mice (n=4) after 4 weeks, whereas no eGFP<sup>+</sup> vWF<sup>+</sup> cells were observed among 616 vWF<sup>+</sup> cell profiles analyzed from negative control apoE<sup>−/−</sup> mice (n=6).

Regenerated ECs After Plaque Disruption Are Derived From the Local Vessel Wall

To investigate whether local cells regenerate plaque endothelium after plaque disruption, we studied healed CB plaques that had been transplanted from apoE<sup>−/−</sup> mice into eGFP<sup>+</sup> apoE<sup>−/−</sup> mice (n=9) and subjected to mechanical plaque disruption 4 weeks before (experiment 4; Figures 1B and 2). We analyzed 1765 vWF<sup>+</sup> cells in serial sections (Figure 7) but found only 1 recipient-derived EC (eGFP<sup>+</sup> vWF<sup>+</sup>). This EC may have originated from the blood
or may simply have been a migrating EC from the contiguous vasculature. Consistently in the reverse experiment in which eGFP<sup>+</sup> apoE<sup>−/−</sup> CB plaques were transplanted into apoE<sup>−/−</sup> mice (n = 5), 75% of vWF<sup>+</sup> cells (436 of 581 analyzed) were eGFP<sup>+</sup> (Figure 8).

**Discussion**

This series of experiments assessed the contribution of circulating EPCs to ECs over intact and disrupted atherosclerotic plaques. Using relevant mouse transplantation models, specific markers for cell type (vWF) and origin (eGFP and Y chromosome), and high-resolution microscopy, we found few, if any, plaque ECs originating from BM-derived or other bloodborne cells. Thus, we could not confirm the prevailing view that circulating EPCs play a significant role in the development of atherosclerosis.

**Natural History of Atherosclerosis**

Atherosclerosis is an inflammatory disease in which endothelial function and regeneration are crucial. Mobilization, circulation, homing, and local differentiation of BM-derived leukocytes play important roles in disease progression, and the cytokines and their receptors responsible for recruitment of leukocytes to atherosclerotic lesions being unraveled.

In contrast, the widespread theory that BM-derived cells contribute to the endothelial lineage in atherosclerosis is founded on a series of indirect observations in humans and mouse models, many of which, in hindsight, can be criticized for a number of methodological problems.

**Circulating EPCs and Atherosclerosis**

Genuine EPCs appear to exist in the blood but are exceedingly rare. Their detection requires long-term culturing under conditions that favor endothelial differentiation, and their identity before culturing and fate in vivo remain unknown. Based on EC-like characteristics acquired during short-term culturing of adherent or replated nonadherent cells, a population of colony-forming mononuclear cells in the human peripheral blood, similar to those described by Asahara et al., were initially believed to be EPCs. The colonies they produce, however, were later shown to be composed mainly of inflammatory and immune cells rather than ECs (reviewed elsewhere). For unknown reasons, the ability of the blood to form such colonies correlates with endothelial function (flow-mediated brachial artery reactivity), atherogenic risk factors, established atherosclerotic disease, and future atherosclerotic events. It has never been shown, however, that these supposed EPCs or any other circulating cells do home, incorporate, and differentiate into mature ECs over established atherosclerotic lesions in humans or in animals.

**Mouse Models Relevant to Atherosclerosis**

Endothelial repair by BM-derived EPCs has been reported in a model of vein graft atherosclerosis and in atherosclerosis-prone regions of arteries. Atherosclerosis in vein isografts differs from atherosclerosis in native arteries. In a recently described mouse model, the vein graft undergoes necrosis with loss of ECs within a few days, and the endothelium subsequently regenerates with ECs of recipient origin. The majority of them may migrate from the contiguous vasculature, but a contribution from BM-derived EPCs was also reported. The true EC phenotype of the BM-derived cells in this study, however, remains debatable because of the reliance on a nonspecific EC marker (Tie-2), as discussed below. The same methodological concerns apply to the recent study focusing on endothelial turnover and repair in atherosclerosis-prone areas in apoE<sup>−/−</sup> mice.

**Cell Therapy in Atherosclerosis**

In a few studies, atherosclerotic mice were treated intravenously with BM- or blood-derived cell populations assumed to contain EPCs. Discrepant effects on atherosclerosis were observed, but most important is the fact that no conclusive signs of homing, incorporation, and endothelial differentiation of the injected cells over atherosclerotic lesions were reported. Considering that until now all of the donated cell populations have been rich in leukocytes, including their progenitors, with an innate desire to adhere to activated endothelium and home to injured tissue, their short-term presence at such sites is to be expected.

**Methodological Challenges: Colocalization of Specific Markers**

In general, reliable identification of the phenotype and origin of a cell requires that 2 specific markers colocalize to a single cell. To avoid false-positive colocalization, small and diffusible markers such as eGFP need to be fixed in situ, and tissue morphology and microscopic examination must provide single-cell resolution. With a thickness of less than a few microns, colocalization of 2 signals to a single EC is particularly demanding. Furthermore, BM-derived cells frequently reside in the subendothelial space in close proximity to ECs (Figure 7e), accentuating the risk of false-positive colocalization if single-cell resolution is not reached.

In the present study, vWF was used as an EC marker. Although vWF is not detectable in all ECs in the mouse, it is...
expressed robustly in larger arteries, restricted to ECs, stored in the endothelium-specific Weibel-Palade bodies, and secreted to the blood.\textsuperscript{38,39} On or within the arterial wall, the only other source of vWF positivity is platelets and matrix-bound vWF. Thus, in arteries of mice, vWF is a specific EC marker when identified within the cytoplasm of nucleated cells, usually with a granular staining pattern, by single-cell resolution microscopy. Two potential limitations pertaining to the sensitivity of this marker must be noted. First, we found few vWF\textsuperscript{+}, elongated, lumen-bordering cells in plaques that by morphology appeared to be ECs; hence, vWF may not be completely sensitive for plaque endothelium. It is important to note, however, that none of these putative ECs identified by morphology were eGFP\textsuperscript{+} in BM-transplanted mice. Second, vWF is not a sensitive marker for microvascular ECs in mice,\textsuperscript{38} but we did not try to assess, and in fact did not observe, plaque neovascularization (angiogenesis) in the present study.

Much of the confusion regarding differentiation of putative EPCs into ECs results from the use of nonspecific EC markers shared by subpopulations of leukocytes such as in vitro adhesion, uptake of acetylated low-density lipoprotein, lectin binding, expression of CD31/PECAM-1, and activity of the Tie-2 promoter.\textsuperscript{25–27,34,40,41} The Tie-2 promoter has been used extensively in BM-transplanted mice to track and prove the participation of BM-derived ECs in neovascularization\textsuperscript{2,3,9,42,43} and endothelial regeneration after mechanical injury.\textsuperscript{4,44} in vein isografts,\textsuperscript{8} and in atherosclerosis-prone areas.\textsuperscript{12} Recent research has shown that Tie-2 is not entirely restricted to the EC lineage but is also expressed by a proangiogenic subpopulation of blood monocytes and tissue macrophages located close to proliferating ECs and easily mistaken for them.\textsuperscript{20,45} Another confounding factor is that the Tie-2–driven reporter β-galactosidase may be endogenously expressed in cells not belonging to the EC lineage, particularly in senescent cells.\textsuperscript{46}

Conclusions

Circulating EPCs rarely, if ever, contribute to the maintenance and regeneration of plaque endothelium in the apoE\textsuperscript{−/−} mouse model of atherosclerosis. These findings do not support the prevailing but untested theory that circulating EPCs play a role in human atherosclerosis.

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Disclosures

None.

References

Clinicai Perspective

The idea that endothelial progenitor cells (EPCs) circulate in blood and contribute to endothelial cell turnover and regeneration in the cardiovascular system has become a widely accepted paradigm. Clinical studies have measured the level of putative EPCs in the blood of patients and found that it correlates inversely with atherogenic risk factors, established atherosclerotic disease, and future atherosclerotic events. This has fostered the theory that circulating EPCs provide protection against atherosclerosis by their innate ability to replace dysfunctional ECs and to regenerate senescent and damaged endothelium. Direct evidence that EPCs contribute to plaque endothelium at all is sparse, however. In the present study, we investigated whether circulating EPCs home and differentiate into endothelial cells in murine atherosclerotic plaques using a series of transplantation techniques, specific markers for cell type and origin, and high-resolution microscopy. We report that endothelial cells in both intact and healed disrupted plaques are replenished by local and circulating EPCs or EPC-like cells. Further, we demonstrate that EPCs contribute to plaque endothelium at all stages of atherogenesis. These findings have important implications for understanding the role of EPCs in human disease and for the development of therapeutic strategies to combat atherosclerosis.
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