UltraRapid Communication

Both Donor and Recipient Origins of Smooth Muscle Cells in Vein Graft Atherosclerotic Lesions

Yanhua Hu, Manuel Mayr, Bernhard Metzler, Martin Erdel, Fergus Davison, Qingbo Xu

Abstract—Smooth muscle cell (SMC) accumulation in the inner layer of the vessel wall is a key event in the pathogenesis of atherosclerosis in vein grafts, but the origin of the cells in these lesions has yet to be shown. Herein, we use animal models of vein grafts in transgenic mice to clearly identify the sources of SMCs in atherosclerosis. Vena cava segments were isografted to carotid arteries between four types of transgenic mice, including SM-LacZ expressing β-galactosidase (β-gal) in vascular SMCs, SM-LacZ/apoE$^{-/-}$, ROSA26 expressing β-gal in all tissues, and wild-type mice. β-gal–positive cells were observed in neointimal and atherosclerotic lesions of all vein segments grafted between LacZ transgenic and wild-type mice. Double staining for β-gal and cell nuclei revealed that about 40% of SMCs originated from hosts and 60% from the donor vessel. This was confirmed by double labeling of the Y-chromosome and α-actin in the lesions of sex-mismatched vein grafts. The possibility that bone marrow cells were the source of SMCs in grafts was eliminated by the absence of β-gal staining in atherosclerotic lesions of chimeric mice. Furthermore, vein SMCs of SM-LacZ mice did not express β-gal in situ, but did so when these cells appeared in atherosclerotic lesions in vivo, suggesting that hemodynamic forces may be crucial for SMC differentiation. Thus, we provide the first evidence of SMC origins in the atherosclerotic lesions of vein grafts, which will be essential for providing insight into new types of therapy for the disease. The full text of this article is available at http://www.circresaha.org. **(*Circ Res*. 2002;91:e13-e20.)**

Key Words: atherosclerosis ■ vein grafts ■ smooth muscle cells ■ cell origin ■ transgenic mouse models

Vein grafts remain the only surgical alternative for many types of vascular reconstruction, but the patency rate is limited due to obliterative stenosis of the grafted vessels.[1,2] The occlusion of the vessel is due to the formation of atherosclerosis-like lesions in the intima, in which smooth muscle cells (SMCs) are the main cellular components.[1,3] It is believed that SMCs in spontaneous atherosclerosis are derived from the media of the artery in response to platelet-derived growth factor (PDGF) released by injured endothelial cells and aggregated platelets. However, this concept is challenged by recent findings demonstrating that other sources of SMCs may contribute to vascular diseases.[4–9] We observed that SMCs in vein grafts appear in the neointima earlier than in the media after cell death, which is an early cellular event in the grafted vessels.[4] Shi et al[5] demonstrated that myofibroblasts in the adventitia of arteries may contribute to neointimal formation in response to endothelial cell injury. In addition, current evidence indicates that bone marrow progenitor cells may be a source of SMCs for transplant arteriopathy,[6] neointimal lesions of injured arteries,[10,11] and hypercholesterolemia-induced atherosclerosis.[12]

Several animal models manifesting lesions resembling neointimal hyperplasia of human vein grafts have been developed,[13,14] but no spontaneous atheromatous lesions in their vein grafts have been observed. Recently, we developed and characterized a new animal model of vein graft atherosclerosis in apolipoprotein E (apoE)–deficient mice.[15] The lesion displayed classical complex morphological features and heterogeneous cellular compositions. Furthermore, transgenic mice expressing LacZ genes controlled by specific SMC or house keeping gene promoters are available.[16–18] These mice express β-galactosidase (β-gal) only in SMCs (SM-LacZ)[16] or most types of cells (ROSA26).[17] When these mice are crossed with apoE knockout mice, which develop spontaneous atherosclerosis,[19] then staining the tissue with X-gal enables the detection of SMC origins in the lesion. Based on this knowledge, we decided to clarify SMC origins in the atherosclerotic lesions of vein grafts because it is a fundamental issue with regard to the pathobiology of atherosclerosis. Using our animal models for vein graft atherosclerosis,[15,20] we performed vein isografts in 4 types of transgenic mice expressing β-gal in different tissues, including SM-LacZ,[16] SM-LacZ/apoE$^{-/-}$, ROSA26, and wild-type mice.[19]

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Materials and Methods

Mice and Vein Graft Procedure

All animal experiments were performed according to protocols approved by the Institutional Committee for Use and Care of Laboratory Animals. Transgenic SM-LacZ mice expressing β-gal under the control of the smooth muscle–specific protein SM22 promoter have been described.[16] The ROSA26 mouse (The Jackson Laboratory, Bar Harbor, Maine) is a β-gal transgenic mouse produced by random retroviral LacZ gene insertion in embryonic stem cells; there are 3 transcription start sites, involving unknown housekeeping gene promoters.[17] ROSA26 mice express β-gal activity in adult tissues. β-gal activity of cells from both mice is mainly localized in the nucleus. ApoE$^{-/-}$ mice (The Jackson Laboratories) were crossed with SM-LacZ mice, and heterozygous offspring were mated to produce apoE-deficient mice expressing β-gal in arterial SMCs (SM-LacZ/apoE$^{-/-}$). All mice used in the present experiment were bred in our laboratories.

All transgenic mice are of strain C57BL/6. Three genotypes of LacZ$^{-/-}$, $^{+/-}$, and $^{+/+}$ mice were identified using The Jackson Laboratory's PCR protocol (primers, 5′-ATCCTCTGCATGGTCAGGTC-3′ and 5′-CGTGGCCTGATTCATTCC-3′). For apoE$^{-/-}$ mice genotyping, a similar protocol was used with primers: oIMR180 5′-GCCTAGCCGAGGGAGAGCCG-3′, oIMR181 5′-TGTGACTTGGGAGCTCTGCAGC-3′, and oIMR182 5′-GCCGCCCCGACTGCATCT-3′. The mice were maintained on a light/dark (12/12 hours) cycle at 22°C, receiving food and water ad libitum.

The vein graft procedure was similar to that described previously.[21,22] Briefly, 3-month-old mice were anesthetized with pentobarbital sodium (50 mg/kg body weight, IP). The vena cava was harvested from the donor. For the recipient, the right common carotid artery was mobilized free from the bifurcation at the distal end toward the proximal, cut in the middle, and a cuff placed at the end. The cuff was made of an autoclavable nylon tube 0.63 mm in diameter outside and 0.5 mm inside (Portex LTD). The artery was turned inside out over the cuff and ligated. The vein segment was grafted between the two ends of the carotid artery by sleeving the ends of the vein over the artery-cuff and ligating them together with the 8-0 suture. The complete grafting procedure required 30 to 40 minutes.

Histology and Immunohistology

The grafts were harvested at 8 weeks postoperatively (6 to 8 mice per group) by cutting the grafted segments from the native vessels at the cuff end. Femurs and tibias were harvested for either section or bone marrow cell preparations. For sections, bones were fixed with 4% phosphate-buffered formaldehyde at 4°C in the presence of EDTA for 1 week. Vessel samples were fixed with 4% phosphate-buffered formaldehyde at 4°C for 24 hours. The grafts were processed by routine histology and embedded in paraffin. Sections (4 μm) begun at the center of the graft were stained with hematoxylin and eosin (HE) for histological evaluation.[23]

For frozen section preparation, vein grafts were harvested, immediately frozen, and stored in liquid nitrogen. The procedure for immunohistological staining was similar to that described previously.[15,21] Briefly, serial 5-μm frozen sections were overlaid with a mouse monoclonal antibody against α-actin (Sigma) labeled with phosphatase. Sections were developed with the appropriate substrate solution (Sigma).

X-Gal Staining

For X-gal staining, frozen mouse aortic and bone sections and cultured cells were fixed in 2% formaldehyde and 0.2% glutaraldehyde in PBS for 5 minutes. The procedure for determining β-gal activity in sections or cultured cells was similar to that described by Sanes et al.[24] Briefly, sections or cultured cells were incubated at 30°C or 37°C for 18 hours in PBS supplemented with 1 mg/mL X-Gal (Sigma), 5 mmol/L potassium ferricyanide, 5 mmol/L potassium ferrocyanide, and 2 mmol/L $MgCl_2$. Sections were rinsed with 3% DMSO in PBS.[25] For double staining of X-gal and SMC α-actin, we performed X-gal staining before SMC α-actin staining. For double staining of β-gal and cell nuclei, we performed X-gal staining before nucleus staining with Hoechst 33258 (1 μg/mL for 1 minute). Positive cells were enumerated under the microscope.

Bone Marrow Transplantation

Donor mice were killed, and femurs and tibias were removed aseptically. Marrow cavities were flushed with Ca,Mg-free Hanks' balanced salt solution (HBSS) (GIBCO-BRL) using a 25-gauge needle attached to a syringe. Single cell suspensions were prepared by repeat pipetting and the cell preparations passed through a nylon mesh to remove particulate matter. Cells were washed twice in HBSS, counted using a hemocytometer, and resuspended at 3×10^7 cells/mL before transplantation. Six- to eight-week-old mice received a lethal dose of whole body x-ray irradiation (950 Rads). The irradiated recipients received 1×10^7 bone marrow cells in 0.3 mL RPMI 1640 by tail vein injection. Vein grafts were performed 4 weeks after bone marrow transfer.

In Situ Hybridization

Vein graft sections and bone marrow smears were air-dried, fixed in methanol/acetic acid, washed in 2×SSC buffer for 10 minutes at 37°C, and dehydrated in graded ethanol. Sections and smeared cells were denatured by immersing them in 70% formamide/2×SSC at 73°C for 4 minutes and dehydrated again in graded ethanol at −20°C. We used the probe pY353B as described by Bishop et al[26] that hybridizes specifically to a series of repetitive sequences on the Y chromosome. The entire plasmid was amplified in bacteria and purified by Maxi Prep (Qiagen). After purification, the probe was nick-translated in the presence of digoxigenin-11-dUTP using a commercial kit (DIG-Nick Translation Mix; Roche).The digoxigenin-labeled Y-probe was denatured in 50% formamide/10% dextran sulfate/2×SSC buffer at 75°C for 5 minutes in the presence of salmon sperm DNA, and hybridized to sections in a humidity chamber at 37°C overnight (30-ng probe per slide). After washing 4 times with TBS, hybridized probe was detected using anti-digoxigenin Fab conjugated with alkaline phosphatase followed by a NBT/BCIP substrate conversion (Rembrand RISH and AP Detection Kit, KREATECH Diagnostics). Positive hybridization of the Y-bearing cells were identified by a small, discrete area of brown precipitate in the nucleus. For double staining of in situ hybridization and SMC α-actin, we performed in situ hybridization before SMC α-actin staining.

Bone Marrow Cell Culture

Harvested bone marrow cells (1×10^6/mL) were plated on 8-well slide chambers in RPMI 1640 supplemented with 10% fetal calf serum and incubated at 37°C in 5% CO_2 for 3 hours. Nonadhered cells were removed using serum-free RPMI 1640. Adhered cells were fixed and stained for X-gal and/or α-actin. A proportion of cultured cells was incubated at 37°C with PDGF-BB (10 ng/mL; Sigma) for 2.5 days, fixed, and stained for X-gal and/or α-actin.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The procedure used for RT-PCR was similar to that described elsewhere.[27] Total RNA was prepared with a RT-PCR Miniprep Kit (STRATAGENE). The following primers were used: SM22 (5′-GCAGTCCAAAATTGAGAAGA-3′ and 5′-CTGTTGCTGCCCATTTGAAG-3′); α-actin (5′-ACGGCCGCCTCCTCTTCCTC-3′ and 5′-GCCCAGCTTCGTCGTATTCC-3′); smooth muscle myosin heavy chain (5′-GACAACTCCTCTCGCTTTGG-3′ and 5′-GCTCTCCAAAAGCAGGTCAC-3′); h1-calponin (5′-GATACGAATTCAGAGGGTGCAGACGGAGGCTC-3′ and 5′-GATACAAGCTTTCAATCCACTCTCTCAGCTCC-3′); GAPDH (5′-CGGAGTCAACGGATTTGGTCGTAT-3′ and 5′-AGCCTTCTCCATGGTGGTGAAGAC-3′).

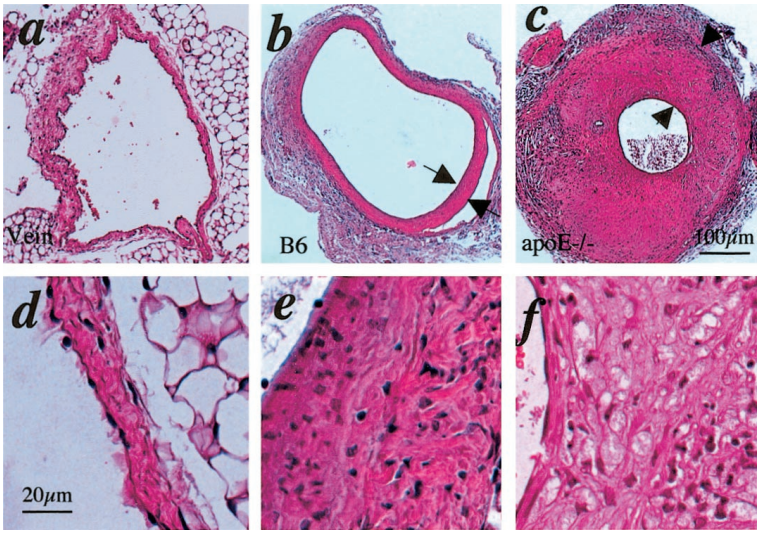

Figure 1. Morphology of neointimal and atherosclerotic lesions of vein grafts in mice. Under anesthesia, vena cava (a and d) of the mouse was removed and grafted into carotid arteries of wild-type (b and e) or apoE$^{-/-}$ (c and f) mice. Animals were euthanized 8 weeks after surgery, and the grafted tissue fragments fixed in 4% phosphate-buffered (pH 7.2) formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin-eosin (HE). Arrows indicate lesions.

Results

Characterization of Lesions

All mice used in the study were of the strain C57BL/6; therefore, the vena cava was isografted into the carotid artery of different animals. Figures 1a and 1d are HE-stained sections from freshly harvested vena cava showing only 2 to 3 layers of cells in the vessel wall. Eight weeks after grafting, more than 20 layers of cells formed neointimal lesions in wild-type mice, which contains abundant cells and matrix protein deposition (Figures 1b and 1e). Interestingly, atherosclerotic lesions with lipid deposition and foam cell formation were observed in vein grafts of apoE-deficient mice (Figures 1c and 1f), which is similar to lesions of vein graft atherosclerosis observed in humans.

Cell composition analysis revealed that about 50% of total cells were SMCs in the neointimal lesions of 8-week vein grafts in wild-type mice,[23] whereas 30% of the cells in atherosclerotic lesions showed α-actin–positive staining in apoE$^{-/-}$ animals.[15] In the present study, we confirmed the presence of SMCs in both neointimal and atherosclerotic lesions of vein grafts in transgenic mice. Figure 2 shows representative sections labeled with anti–α-actin antibody. Only 1 or 2 layers of positively stained SMCs were found in normal veins (Figure 2b), whereas abundant SMCs were present in neointimal lesions (Figure 2c). Less SMCs in atherosclerotic lesions of vein grafts in apoE$^{-/-}$ mice were observed compared with neointimal lesions (Figure 2d versus 2c). Figure 2a is a negative control showing no positive staining.

Recipient and Donor Origins of SMCs

To determine whether recipients contribute to the source of neointimal and atherosclerotic SMCs of vein grafts, vein segments from wild-type and apoE$^{-/-}$ mice were grafted into SM-LacZ transgenic and SM-LacZ/apoE$^{-/-}$ mice, respectively. Eight weeks after grafting, sections of normal veins and vein grafts were analyzed for β-gal enzymatic activity using X-gal substrate. As expected, sections of wild-type and apoE$^{-/-}$ veins showed no β-gal activity (Figures 3a and 3b), whereas carotid arteries of SM-LacZ and SM-LacZ/apoE$^{-/-}$ mice had strong β-gal staining (Figures 3c and 3d). Interestingly, β-gal activities were detected in the vessels of wild-type and apoE$^{-/-}$ mice isografted into the carotid arteries of SM-LacZ (Figure 3e) and SM-LacZ/apoE$^{-/-}$ (Figure 3f) mice, indicating that neointimal and atherosclerotic cells were derived, at least in part, from recipients.

As shown above, arterial SMCs of SM-LacZ mice had a strong positive staining for β-gal, but an absence of staining was seen in sections of veins from either SM-LacZ or SM-LacZ/apoE$^{-/-}$ animals (Figures 4a and 4b). Surprisingly, many β-gal–positive cells were found in neointimal (Figure 4c) and atherosclerotic lesions (Figure 4d) of vein grafts donated by SM-LacZ and SM-LacZ/apoE$^{-/-}$ mice. These

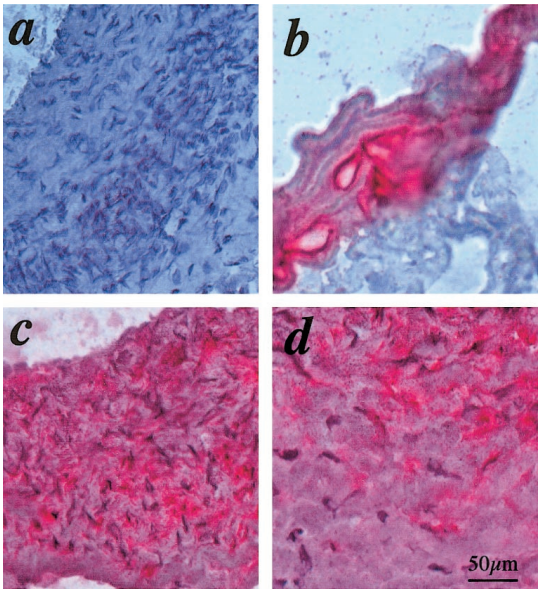

Figure 2. Immunohistochemistry of vein grafts. Sections derived from normal vein (b), vein grafts of wild-type (a and c), and apoE$^{-/-}$ (d) mice at 8 weeks were labeled with normal mouse serum (a; control) or a monoclonal antibody against α-actin conjugated with alkaline phosphatase (b through d) and developed with the substrate. Counterstaining (blue) with hematoxylin was performed. Note that red staining indicate positive cells.

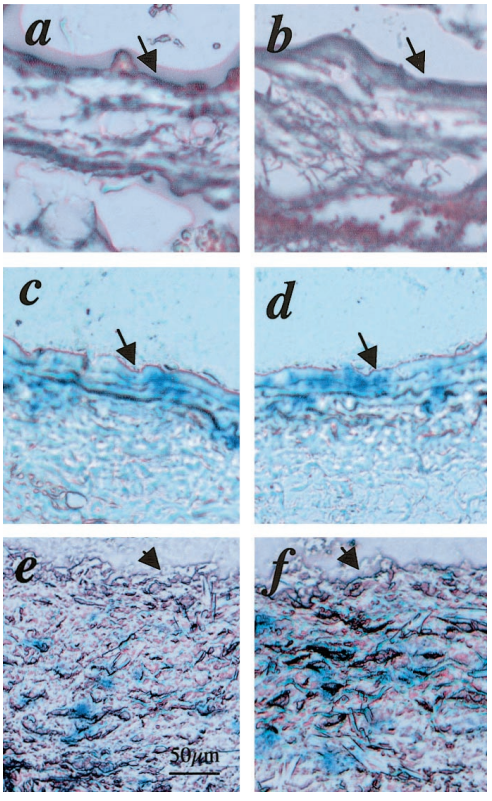

Figure 3. Contribution of recipients to SMC accumulation in lesions. Sections of vessel segments were incubated with a substrate X-gal as described in the Materials and Methods. Blue color indicates β-gal activity. a, Vena cava from a wild-type mouse; b, Vena cava from an apoE$^{-/-}$ mouse; c, Carotid artery from a SM-LacZ mouse; d, Carotid artery from a SM-LacZ/apoE$^{-/-}$ mouse; e, 8-week vein graft from a wild-type mouse grafted into SM-LacZ mouse; f, 8-week vein graft from an apoE$^{-/-}$ mouse grafted into a SM-LacZ/apoE$^{-/-}$ mouse. Arrows indicate the surface of the intima.

results suggest that SMCs from donor vessels could partially contribute to the lesions of vein grafts.

To semiquantify SMCs in the atherosclerotic lesions of vein grafts contributed by recipients and donors, respectively, we performed a double staining using X-gal and the nuclear fluorescence dye Hoechst 33258. Because β-gal is localized in the nucleus, the blue color of β-gal labeling and the fluorescence nucleus staining can be easily distinguished by UV filter and visual light. Even under the visual light both labels can be seen as shown in Figure 5a, in which the nuclei appeared as brighter points. Using this technique, we enumerated total cells and the β-gal-positive cells of vein graft atherosclerosis, respectively, and calculated the percentage of double-positive cells. Data shown in Figure 5b, left panel, summarize the results of means from 6 animals per group. When the data were recalculated by pooling the double-positive cells from recipients and donors as 100%, SMCs were about 40% and 60% derived from recipients and donors, respectively (Figure 5b, right panel). These results indicate that both recipients and donors contribute to the SMC accumulation during the formation of vein graft atherosclerosis.

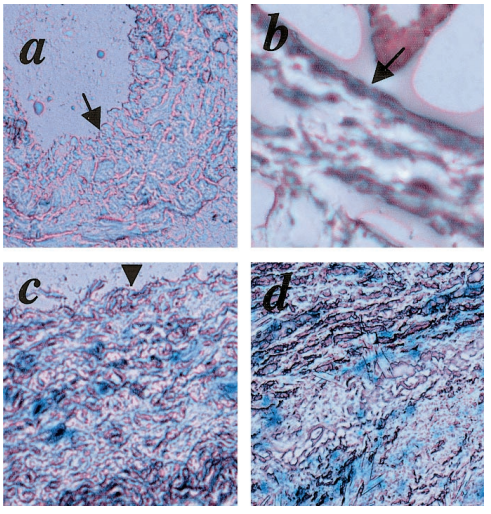

Figure 4. Contribution of donors to SMC accumulation in lesions. Sections of vein segments were incubated with a substrate X-gal as described in the Materials and Methods. Blue color indicates β-gal activity. a, Vena cava from a SM-LacZ mouse; b, Vena cava from a SM-LacZ/apoE$^{-/-}$ mouse; c, 8-week vein graft from a SM-LacZ mouse grafted into a wild-type mouse; d, 8-week vein graft from a SM-LacZ/apoE$^{-/-}$ mouse grafted into an apoE$^{-/-}$ mouse. Arrows indicate the surface of the intima.

Confirmation of Recipient and Donor Origins

To confirm these results, we performed vein grafting in a sex-mismatched manner, and Y-chromosome was detected in sections of vein grafts using in situ hybridization. As expected, Y-chromosome–positive labeling in male liver was

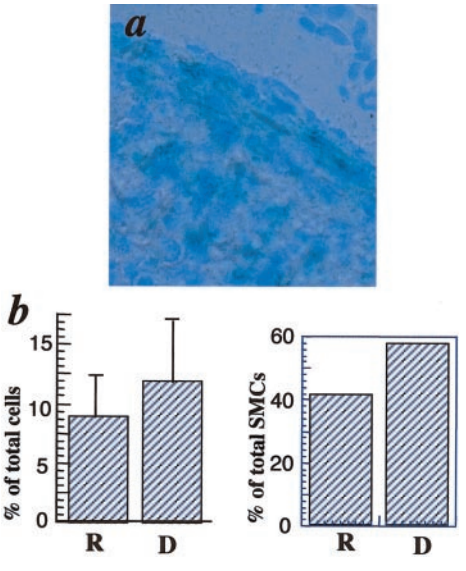

Figure 5. Semiquantification of SMCs in lesions. Sections of 8-week vein grafts from SM-LacZ/apoE$^{-/-}$ mice were double stained for β-gal and the nucleus. Positive cells were counted under the microscope in a 200× field. Four fields per section were selected, and 6 animals per group were included. a, Representative section of vein graft from a SM-LacZ mouse grafted into a wild-type animal stained with X-gal and Hoechst 33258. b, left, Summary data of mean±SD from 6 animals; right, data of SMCs against total numbers of SMCs, which include both origins (100%) from recipients and donors. R indicates recipients; D, donors.

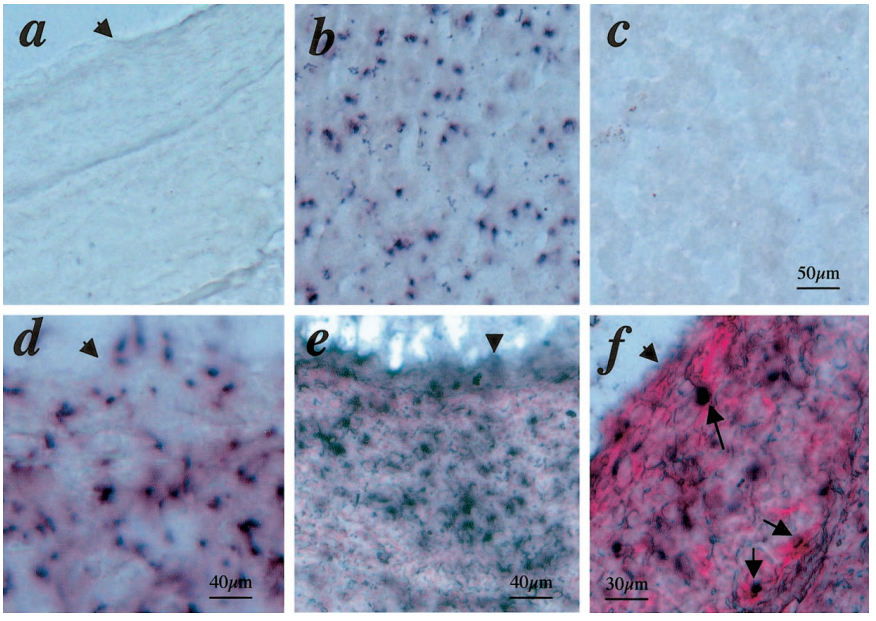

Figure 6. Y-chromosome detection in sex-mismatched vein grafts. In situ hybridization for Y-chromosome was performed for all sections as described in the Materials and Methods. a, Male vein grafted to a female mouse as a negative control omitted Y-chromosome probe for in situ hybridization; b, Male liver section; c, Female liver section; d, Male vein grafted into a female mouse; e, Female vein grafted to a male mouse; f, Male vein grafted to a female mouse and double staining for α-actin (red) and Y-chromosome (brown). Arrowheads indicate the surface of the lesions. Arrows indicate examples of double positive cells.

seen, but not in females (Figures 6b and 6c). A negative control missing the cDNA probe showed no staining in sections of a vein graft (Figure 6a), indicating that the technique was consistent. Sex-mismatched vein grafts of mice showed positive labeling in vein grafts donated by both male and female mice (Figures 6d and 6e), confirming the contribution by both recipients and donors. Double staining for SMC marker α-actin and Y-chromosome in sections of a male-donated vein graft demonstrated that a portion of Y-chromosome–positive cells were SMCs (Figure 6f).

Because ROSA26 mice were used to trace the cell origins in other types of arteriosclerosis, eg, transplant vasopathy,[6] we also used these animals for vein isografting. Vein sections from ROSA26 mice had a strong positive staining for β-gal (Figure 7a), but not wild-type animals (Figure 7c). Interestingly, β-gal–positive cells were observed in the sections of neointimal lesions from veins isografted between ROSA26 and wild-type mice (Figures 7b and 7d). A population of these β-gal–positive cells were positively stained for α-actin (data not shown). Again, these results confirm that SMCs in lesions were derived from both donors and recipients.

No Evidence for Bone Marrow Origins of Lesional SMCs

A proportion of SMCs was identified as originating from the recipients; therefore, it would be important to determine the possible contribution of bone marrow cells. In the present study, chimeric mice were created by bone marrow transplantation after irradiation. Almost all bone marrow cells with nuclei from B6/ROSA26 chimeric mice were stained blue (β-gal$^+$; Figure 8a) although no β-gal activity was seen in bone marrow donated by wild-type mice (Figure 8b). Over 95% bone marrow cells from sex-mismatched female chimeric mice were positively stained for Y-chromosome as identified by in situ hybridization (Figure 8c), whereas no staining was observed in the female bone marrow cells (Figure 8d). Thus, the efficiency of bone marrow transplantation in our chimeric mice was high.

There is evidence that bone marrow cells can differentiate into SMC-like cells.[28,29] To confirm whether the bone marrow cells of SM-LacZ mice can also differentiate into SMCs, cells were cultivated in the presence of PDGF-BB. This growth factor has been shown to effectively stimulate stem cells to differentiate into SMCs.[27] Interestingly, a population of bone marrow cells from SM-LacZ mice showed β-gal positivity in response to PDGF-BB (Figure 9a), and negative staining in the absence of PDGF-BB (Figure 9b). α-Actin staining revealed that a population of bone marrow cells was positive in response to PDGF-BB (Figure 9c), but not in cells

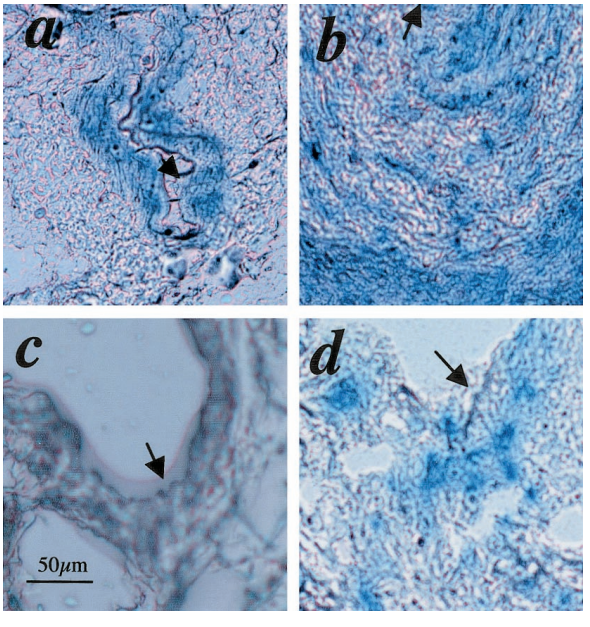

Figure 7. β-gal–positive cells in lesions of vein grafts in ROSA26 mice. Sections from freshly harvested veins of ROSA26 (a) and wild-type (c) and 8-week vein grafts (b and d) were stained for β-gal activity. b, ROSA26 vein grafted to a wild-type mouse; d, wild-type vein grafted to a ROSA26 mouse. Arrows indicate the surface of the intima.

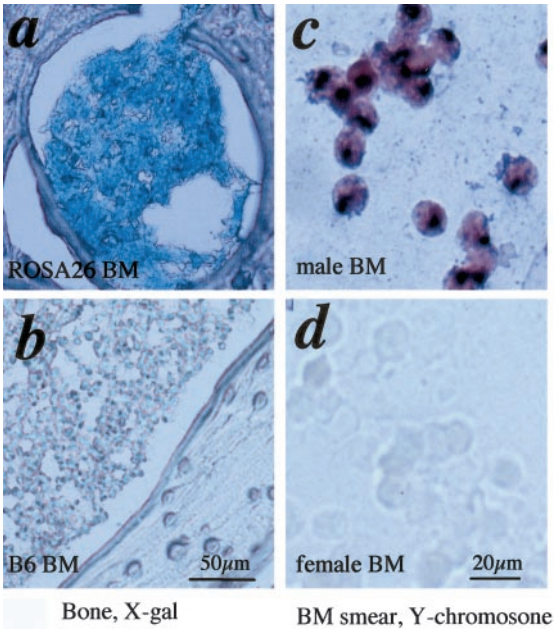

Figure 8. Creation of chimeric mice. Femurs of the chimeric mouse with ROSA26 (a) or wild-type (b) bone marrow (BM) transplantation after irradiation were harvested, sectioned, and stained for X-gal, in which positive cells were stained blue. Bone marrow cells harvested from chimeric mice that had received sex-mismatched marrow cells were prepared for smears and stained by in situ hybridization for the Y-chromosome (c and d). Y-chromosome–positive cells are stained brown.

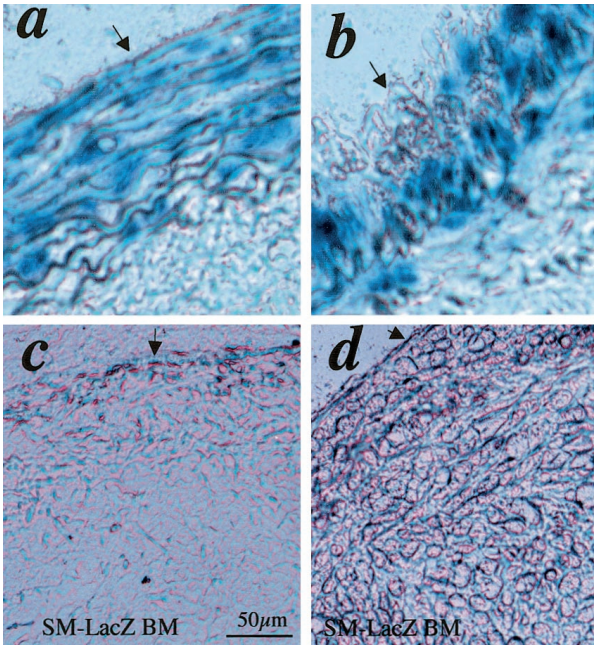

Figure 10. Non-bone marrow origin of SMCs in atherosclerotic lesions. β-gal staining for aortic sections from SM-LacZ (a), SM-LacZ/apoE$^{-/-}$ (b) mice, which served as bone marrow donors. c, Wild-type vein grafted to a chimeric mouse with SM-LacZ bone marrow cells; d, Wild-type vein grafted to a chimeric mouse with SM-LacZ/apoE$^{-/-}$ bone marrow cells. Sections were stained for X-gal. Arrows indicate the surface of the intima.

without PDGF-BB stimulation (Figure 9d). Concomitantly, RT-PCR analysis demonstrated that SM22, α-actin, calponin, and myosin heavy chain were highly expressed in bone marrow cells stimulated by PDGF-BB, but not untreated cells (Figure 9e). These findings suggest that bone marrow cells from SM-LacZ mice can differentiate into SMC-like cells and express β-gal in response to PDGF-BB stimulation.

To create chimeric animals, bone marrow cells from SM-LacZ and SM-LacZ/apoE$^{-/-}$ mice were harvested and transferred to irradiated wild-type mice. Aortic sections from bone marrow donors had strong β-gal positivity (Figures 10a and 10b). When vein segments from wild-type mice were grafted into chimeric mice with bone marrow derived from SM-LacZ (Figure 10c) and SM-LacZ/apoE$^{-/-}$ (Figure 10d) mice, no β-gal activity was seen in atherosclerotic lesions of vein grafts (Figures 10c and 10d). Six animals per group were used, a series of sections (20 to 60 sections) examined, and no β-gal–positive cells found. Thus, bone marrow cells are unlikely to be a source of SMCs in the atherosclerotic lesions of vein grafts.

Discussion

The source of SMCs in neointimal and atherosclerotic lesions of vein grafts is a fundamental issue in understanding the pathogenetic interactions of cells involved in the disease process. In the present report, we provide the first evidence that neointimal and atherosclerotic SMCs of vein grafts

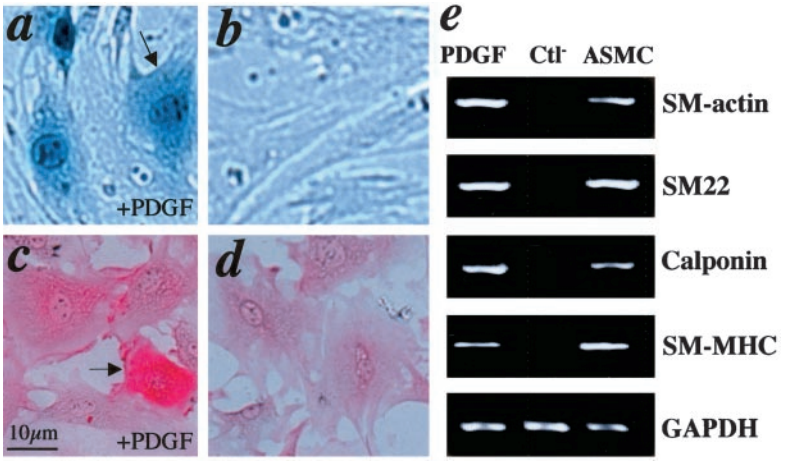

Figure 9. Bone marrow cells can differentiate into SMC-like cells. Bone marrow cells from chimeric mice were harvested and cultivated in RPMI 1640 supplemented with 10% FCS for 3 hours. Cells were either harvested (b and d) or continuously cultivated for 2.5 days in serum-free medium in the presence of human PDGF-BB (10 ng/mL; a and c). Cells were stained for X-gal (a and d) and α-actin (c and d). Note X-gal stained blue and α-actin red, respectively. e, RT-PCR data showing a lack of SMC marker proteins in untreated bone marrow (BM) cells. ASMC indicates arterial smooth muscle cells.

originated from recipients and donor vessels, as identified by directly SMC SM22-driven β-gal expression. We demonstrate that about 40% of SMCs in atherosclerotic lesions were derived from recipients and 60% from grafted vessels. These data establish the heterogeneous origins of SMCs in both neointimal and atherosclerotic lesions of vein grafts. Thus, our findings are crucial for understanding the pathogenesis of vein graft atherosclerosis, and in establishing new strategies for therapeutic intervention in this disease.

Recipient SMC Origins

About 40% of total SMCs in lesions were identified as of recipient origin. Where are these SMCs derived from? One possibility is that medial SMCs of recipient anastomosed arteries migrate into the graft. Another possibility is that bone marrow cells differentiate into SMC progenitor cells, which were proposed to be a source of smooth muscle–like cells in lesions of transplant arteriopathy[6] and neointimal lesions of injured arteries.[11] However, our findings using chimeric mice demonstrated that neointimal SMCs were not derived from bone marrow cells, because of the complete absence of β-gal activity in the lesions of vein grafts in mice expressing smooth muscle–β-gal in bone marrow cells. The question arose as to whether some bone marrow progenitor cells resistant to irradiation were present in recipients, which subsequently differentiated into SMCs without β-gal–positive staining. Our data should exclude this possibility, because it was difficult to find negatively stained cells in transplanted β-gal–positive bone marrow; either by Y-chromosome in situ hybridization or β-gal staining, indicating that bone marrow was completely replaced by β-gal expressing cells. It was possible that transferred bone marrow cells carrying SM-LacZ gene lost the ability to express β-gal. However, our data suggest that this was not the case, because about 10% of transferred bone marrow cells in culture were β-gal–positive in response to PDGF-BB stimulation. This indicates that SM-LacZ bone marrow cells can differentiate into SMC-like cells expressing SM22, α-actin, calponin, and myosin heavy chain. Although there was such potential in transferred bone marrow cells, no β-gal–positive cells were found in the lesions of vessels grafted to mice with SM-LacZ bone marrow. Therefore, bone marrow is unlikely to be a source of SMCs in atherosclerosis.

Finally, circulating progenitor cells might be derived from other organs, eg, pericytes in the microvasculature.[30,31] These microvessels are able to generate new vessels after injury or stimulation, indicating a potential for angiogenesis. Even liver and spleen might be also a source of SMC progenitor cells, because evidence indicates that adult-derived stem cells from the liver could become myocytes in the heart.[32] Furthermore, Frid et al[33] reported that mature endothelial cells can transdifferentiate into SMCs as defined by expression of α-SM-actin, SM22α, calponin, and SM-myosin, which may be an additional source of SMCs. To investigate this hypothesis further studies will be needed to confirm the presence of SMCs in the circulation and to determine their origins.

Donor SMC Origins

About 60% of total SMCs in lesions are derived from donor vessels as identified by double staining for β-gal and nuclei in sections of vein grafts in SM-LacZ mice, which was confirmed by in situ hybridization for Y-chromosome and ROSA26 animals. Obviously, cells in the grafted vessel could contribute to SMC accumulation forming atherosclerotic lesions, ie, medial SMCs and/or adventitial myofibroblasts. Although it is not clear concerning the exact mechanism of SMC origins from donor organ, there are two possibilities: (1) a local progenitor cell might be activated and differentiated into SMCs where they proliferate. Support for this concept is the data identifying the presence of progenitor cells in a variety of organs.[34] (2) The remaining SMCs of the donor vessel could dedifferentiate and proliferate to constitute the lesions. Further investigations clarifying the impact of each source contributing to SMC origins are being carried out in our laboratory.

It has been known that medial SMCs in veins of SM-LacZ mice do not express β-gal proteins in vivo, but a population of SMCs in atherosclerotic lesions from vein grafts donated by SM-LacZ mice does. This phenomena is similar to that found in veins that do not develop atherosclerosis, but accelerated lesions can be seen in veins grafted to arteries where such veins must bear an increased mechanical force.[35] This led us to consider the contribution of mechanical stress on LacZ gene expression controlled by a SM22 promoter. We demonstrated that mechanical stress resulted in β-gal induction in cultured SMCs from the vena cava, indicating that mechanical stress stimulates venous SMCs to produce proteins that are essential for SM22 promoter-controlled LacZ gene expression (data not shown). Others demonstrated that mechanical stress resulted in smooth muscle cell differentiation into a contractile phenotype.[36,37] Thus, 60% of SMCs in atherosclerotic lesions could originate from the vein grafts, which express SM22-controlled β-gal in response to elevated blood pressure.

In summary, we provide the evidence that both donor and recipient contribute to SMC accumulation in neointimal and atherosclerotic lesions of vein grafts, and that bone marrow cells are unlikely to be a source of SMCs in the lesions. These findings indicate the pathogenesis of vein graft atherosclerosis differs from other types of atherosclerosis, including transplant arteriosclerosis, restenosis after injury, and hypercholesterolemia-induced atherosclerosis. In those reports, authors believe that bone marrow cells might contribute to SMC formation in lesions. Thus, our results suggest that different targets have to be considered when we treat vein graft atherosclerosis versus other types of vascular diseases.

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