Neointima Formation
A Local Affair
Virginia J. Hoglund, Xiu Rong Dong, Mark W. Majesky

Where do intimal smooth muscle cells (SMCs) come from? For many years, the idea that intimal SMCs originated from the underlying media went unchallenged.1 Then, reports2,3 began to appear that up to half of the SMCs in the intima of atherosclerotic plaques and injured arteries arose from circulating progenitor cells of bone marrow origin. This new view of intimal SMC formation was potentially important because it raised the possibility of the new class of therapeutic targets for intervention in the process of restenosis based on a bone marrow derivation of intimal SMCs. However, as other laboratories began to follow up on these intriguing initial reports, a long-term contribution of bone marrow–derived cells to intimal tissue became less tenable.4,5 In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, a careful and detailed study by Daniel et al6 seems to leave little or no room for a role of bone marrow–derived cells as progenitors for the intimal SMCs and endothelial cells that are stable residents of a mature neointima that forms after acute vascular injury.

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Long-Term Analysis of Neointimal Formation
Daniel et al6 transplanted bone marrow from enhanced green fluorescent protein (EGFP)–positive (EGFPpos) mice into lethally irradiated wild-type C57Bl/6 mice, allowed 12 weeks for stable engraftment, performed wire injury to the femoral artery, and then recovered injured tissues from 3 days to 16 weeks after injury. The extended time course is important because most studies of neointimal formation in this model usually stop at 4 weeks (occasionally at 8 weeks) after vascular injury. Daniel et al observed rapid accumulation of EGFPpos cells in neointimal tissue that peaked in the first 2 weeks after injury and accounted for up to 68% of the total cells in the neointima. However, as cells expressing differentiated SMC markers began to accumulate in the neointima, the number of EGFPpos cells in the neointima became markedly diminished. By 16 weeks after injury, only 2% of neointimal cells were EGFPpos and few, if any, EGFPpos neointimal cells expressed the definitive SMC marker proteins calponin or SM myosin heavy chain (Figure). Likewise, few, if any, of the CD31pos endothelial cells in the neointima could be assigned a bone marrow origin by 16 weeks after injury. The researchers6 conclude that the contribution of marrow-derived cells to neointimal formation is best viewed as an early paracrine activity that diminishes with time and that there is little, if any, long-term contribution of marrow-derived cells to neointimal formation.
derived progenitors to the endothelial or SMC components of the long-standing neointima.

**Experimental Design and Technical Considerations**

Several technical and experimental considerations are important in evaluating why the results of Daniel et al.6 reach conclusions that significantly differ from those of earlier reports.2,3 One is the use of high-resolution confocal microscopy and deconvolution analysis of z-axis image stacks. These methods reduce the likelihood of false-positive assignments for EGFPpos cells that appear to express SMC maker proteins that are actually expressed by closely apposed cells in tissue cross sections. A second technical consideration is that Daniel et al found that rapid fixation with 4% paraformaldehyde was necessary to prevent leakage of the EGFP marker protein from a cell that expressed it in vivo to a neighboring cell that did not.6 If too much time was allowed before fixation, cells in the tissue began to release EGFP, most likely through cell lysis, and the fluorescent marker diffused to neighboring binding sites associated with otherwise EGFP-negative (EGFPneg) cells. In addition, an important feature of their experimental design was that the time course was extended up to 16 weeks after arterial injury. This extended time course allowed for a clear distinction between an early inflammatory phase occurring in the first 2 weeks after injury (when the neointima contained a large component of EGFPpos/monocyte+macrophage-2 (MoMa2)pos macrophages) and a late mature phase from 4 to 16 weeks (when EGFPpos cells were lost and EGFPneg cells expressing SMα-actin and the definitive SMC marker proteins [calponin and SM myosin heavy chain] became abundant) (Figure). The results of the study by Daniel et al strongly suggest that the role of bone marrow–derived cells in neointimal formation is primarily a transient paracrine one rather than as a precursor for transdifferentiation into long-term resident endothelial cells and SMCs in neointimal tissue.

**Origins of Neointimal SMCs**

What then is the origin of SMCs in the long-standing neointima? In simpler times, the finding that bone marrow–derived progenitors do not serve as a significant source of neointimal SMCs would lead to the familiar conclusion that medial SMCs are the source of intimal SMCs. Except in extreme models of transplant graft rejection, in which essentially all of the medial cells in the graft are lost through cell death, there is little argument that at least some neointimal SMCs originate in the injured media. However, the media is not the only source of cells from which the injured artery wall can build a neointima. Over the years, there have been occasional reports about a possible role for the adventitia in neointimal formation. For many investigators, these reports were seen as inconsistent with existing dogma and their conclusions were frequently ignored. However, it is hard to deny that the adventitia is highly responsive to most forms of arterial injury. Indeed, there appears to be a continuous communication between the endothelium and the adventitia via transmural mediators whose molecular identity has not yet been characterized. For example, Scott et al7 reported that most proliferating cells in porcine coronary arteries subjected to overstretch injury were found in the adventitia. Injections of bromodeoxyuridine given between days 2 and 3 after injury showed that proliferating adventitial cells can migrate into the neointima, where they were found by day 14 after injury.7 Similar results were reported by Shi et al8 using a saphenous vein graft model. Likewise, in a rat carotid balloon injury model in which adventitial fibroblasts are labeled in vitro with a retrovirus expressing β-galactosidase and then introduced into the carotid adventitia immediately after injury, β-galactosidase–positive cells were found in the injured media at 5 days and in the neointima at 7, 10, and 14 days after injury.9 A review of the literature in 2001 led Sartore et al10 to hypothesize that in response to adult vascular injury, activation of adventitial fibroblasts is, at least in part, reminiscent of a developmental program that invests medial SMCs around newly forming blood vessels.

**Formation of Adventitia in Vascular Development**

The suggestion of a link between mechanisms used to construct artery walls in the embryo and the formation of a neointima in adult vessels raises a number of intriguing questions, particularly with respect to the adventitia. It is well documented that hypertensive remodeling of the hypoxic pulmonary artery results in dramatic wall thickening, including the formation of additional smooth muscle layers. These additional layers form on the adventitial side of the artery wall and may well use SMCs that originate from local progenitors that are natural residents in the adventitia.11 Similarly, in mice haploinsufficient for tropoelastin, additional alternating layers of SMCs and elastic fibers are formed on the adventitial side of the artery wall during the late stages of embryogenesis (around E15.5 to E18.5).12,13 These new SMCs are formed well after the initial cohort of SMC progenitors from the cardiac neural crest and other early embryonic sources have completed investment of artery walls from E10.5 to E14.5.14 A potential source of SMCs that are added to the outside of the artery wall was identified by Hu et al15 and confirmed by Passman et al.16 These studies showed that a population of stem cell antigen 1 (Sca1)+positive cells in the adventitia (AdvSca1) could be isolated by immunomagnetic selection or fluorescence-activated cell sorting for Sca1 expression and found that a significant fraction of these progenitor cells differentiated into SMCs in vitro. These AdvSca1 progenitor cells clustered in the border region between the media and adventitia.16 When transplanted to the outside of a vein graft, AdvSca1 cells were found in the media at 2 weeks and in the neointima at 4 weeks after transplantation, where they composed approximately 20% of the total neointimal cell population.15 AdvSca1 cells normally appear at approximately E16.5 to E17.5 in the aortic adventitia and then are found throughout the arterial system.16 They are not derived from the bone marrow.15 Their initial appearance and/or survival in the embryonic aortic adventitia is dependent on sonic hedgehog signaling in the adventitial layer.16 In this same border region between the media and adventitia of human internal thoracic artery specimens, a population of CD34pos/platelet-endothelial cell adhesion
molecule-1 (PECAM-1)\(^{\text{EGFP}}\) cells is found; this population can form capillary-like microvessels when segments of human internal thoracic arteries are explanted in an aortic ring assay.\(^{17}\)

**Summary**

Taken together, these reports suggest that the arterial adventitia contains a resident population of vascular progenitor cells with the capability to differentiate into SMCs and to migrate from the adventitia to the developing neointima. Indeed, Daniel et al\(^{6}\) identify a highly proliferative fraction of Sca1\(^{\text{pos/CD34pos}}\) cells in the femoral artery adventitia that was EGFP\(^{\text{neg}}\) and, therefore, locally derived. Moreover, at 6 weeks after wire injury, individual Sca1\(^{\text{pos/CD34pos}}\) cells were found within the media, apparently migrating from the adventitia to the neointima, similar to the findings of Hu et al.\(^{15}\) The study by Daniel et al\(^{6}\) provides further support for the conclusions of Wagers et al\(^{14}\) that bone marrow–derived hematopoietic stem cells exhibit little or no plasticity for transdifferentiation into the principle cell types that make up the vessel wall (endothelial cells and SMCs). The unique properties of intimal SMCs argue for a unique origin for these cells.\(^{19}\) The studies of Bentzon et al\(^{3}\) and Daniel et al, among those of other researchers, suggest that an origin of intimal SMCs from circulating progenitors of bone marrow origin is highly unlikely. Persistent reports of adventitial cell proliferation after arterial injury.\(^{6, 7}\) the movement of these cells into the intima,\(^{7, 9, 15}\) and the formation of a vascular progenitor cell niche at the border between the media and the adventitia in embryonic development\(^ {15–17}\) argue for a closer look at the roles played by adventitial cells in the formation of a neointima and in the pathogenesis of intimal disease.

**Acknowledgments**

We thank our colleagues Jenna Regan, PhD, and James Faber, PhD, at the University of North Carolina; and Alexander W. Clowes, MD, Mary Weiser-Evans, PhD, Joseph M. Miano, PhD, and Stephen M. Schwartz, MD, PhD, for their helpful discussions.

**Sources of Funding**

This study was supported by American Heart Association Fellowship 09PRE2060165 (Hoglund); grants HL-93594 and HL-19242 from the National Institutes of Health (Dr Majesky); and the Curriculum in Genetics and Molecular Biology Training Program, University of North Carolina, Chapel Hill.

**Disclosures**

None.

**References**


**Key Words:** restenosis • smooth muscle cell • adventitia • bone marrow
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Arterioscler Thromb Vasc Biol. 2010;30:1877-1879
doi: 10.1161/ATVBAHA.110.211433
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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