Perspectives Series: Cell Adhesion in Vascular Biology

Smooth Muscle Migration in Atherosclerosis and Restenosis

Stephen M. Schwartz
Department of Pathology, University of Washington, Seattle, Washington 98195

Lesions of atherosclerosis are only found in the intima, and the smooth muscle cells (SMC)[1] comprising these lesions are clonal. These two simple facts imply that formation of the intima, presumably by migration of cells from the media, is a key event in the early stages of atherosclerosis. There is also a lot of interest (1), although less data, in the possibility that smooth muscle migration and proliferation are critical in later stages of the disease and, particularly, in restenosis after angioplasty. This review will discuss possible critical roles for migration in the origin and progression of atherosclerotic lesions.

Diversity of smooth muscle

While special properties of the intimal SMC must underlie the clonal localization of atherosclerotic lesions, all SMC have very diverse origins. SMC investing both blood vessel and gut arise locally from the surrounding mesenchyme (1, 2). These diverse origins may imply differences in lineage that could be important to the pathogenesis of diseases of SMC. For example, mesenchyme of head and neck, including vascular smooth muscle, is derived from ectoderm. Cultured mesectodermal SMC have unique properties including higher synthesis of elastin (2), implying that there are genetic differences between mesodermal and mesectodermal smooth muscle. Such differences in SMC lineage could explain the localization of atherosclerotic lesions or even the genetic basis for supravalvular aortic stenosis (3). There is abundant evidence that intimal SMC also have a unique phenotype (1).

Investment of blood vessels by smooth muscle

The first hints of the factors involved in the recruitment of SMC come from recent studies of knockout mice. Deletion of the PDGFβ receptor leads to microvascular rupture due to a lack of pericytes (4). This morphogenic defect suggests that PDGF-β induces pericytes to arise by migration from the SMC of the larger vessels. However, a recent study with chimeric mice made from PDGFβR knockout and wild-type cells suggests that PDGFβR has an even wider role. In animals chimeric for this receptor, all types of muscle show a marked enrichment in the wild-type cells having the PDGFβR. Thus, it is likely that PDGF-β plays a critical role in some early step of the recruitment of muscle progenitors, perhaps by inducing migration of cells from the primitive mesenchyme. This effect may appear again in adult animals where PDGF appears to play a key role in migration of medial SMC into the neointima after vascular injury (1).

Intimal formation

The first point is that the SMC of atherosclerotic lesions are comprised of clones in the intima. Murry et al. (5) have demonstrated recently that the atherosclerotic clone is located in the SMC of the fibrous cap itself, however, smaller clones are also present in normal intima. Thus, clonality could result from developmental expansion of rare cells in the intima as suggested previously by Velican and by Thomas (cited in reference 1). The clonal origins of intimal cells could reflect trapping of rare SMC during formation of the internal elastic lamina, migration of rare cells across the internal elastic lamina at later times, or, as originally proposed by Benditt, a mutation that for some reason only occurs in intimal cells. In any case, clonality suggests that migration of SMC from the media to a location between the endothelium and the media is a necessary step in formation of these lesions. Given the evidence for clonal expansion it seems unlikely that the migration event occurs as a response of the usual sort studied in animal models of arterial injury (below).

Before discussing the role of migration of medial SMC in the spontaneous formation of the intima, we should consider a recent and controversial finding that raises another possible explanation for intimal formation. deRuiter et al. (6) present evidence that endothelial cells can delaminate and move into the subjacent vessel wall during vascular development. Markwald has described something quite similar for the origin of cells in endocardial cushions (2). The origin of such unique SMC, moreover, may not be restricted to the local endothelium. A number of studies in the transplantation and marrow literature suggest that the peripheral blood contains CD34 positive cells with potential to form several cell types, including SMC, as well as endothelial cells (7).

Whatever the source of intimal SMC, intimal formation occurs spontaneously before birth in the ductus arteriosus and in most arteries after birth. Morphologic studies imply that this occurs by migration from the preexisting media (1, 2). Essentially nothing is known about the mechanisms controlling this potentially critical early event in the origins of atherosclerotic lesions.

In contrast, pathological intimal formation has also been seen as a response to many different kinds of injury, ranging

Address correspondence to Stephen M. Schwartz, Department of Pathology, University of Washington, Seattle, WA 98195. Phone: 206-543-0258; FAX: 206-685-3662.

Received for publication 7 May 1997.

1. *Abbreviation used in this paper:* SMC, smooth muscle cells.

J. Clin. Invest.

0021-9738/97/06/2814/04 $2.00
Volume 99, Number 12, June 1997, 2814–2817

from trauma, to lipid feeding, and especially transplant rejection (1). Mechanistic data, however, are almost all confined to one model, the rat carotid artery.

Neointimal formation after injury

The initial response to injury in the rat model is SMC proliferation in the media. This initial proliferative wave must have something to do with the final formation of the intima since antiproliferatives, including antisense directed at genes required for proliferation, have no effect on rates of migration across the internal elastic lamella but do inhibit the final number of cells that migrate (8). Nothing is known about why this initial proliferative event is important. However, intimal cells show loss of the contractile proteins that characterize medial SMC, suggesting that medial cells must undergo a marked change in cytoskeleton in order to migrate. Similar loss of contractile structure is seen in other muscle cells, including skeletal muscle cells, migrating into wounds and it seems quite possible that migration of any differentiated cell into a wound requires dedifferentiation to a motile form.

Migration, after this initial proliferation, is controlled by a redundant set of molecules including PDGF, angiotensin II, TGF-β, and FGF. However, even when inhibitors have been used, after several weeks an intima still appears. Thus, low levels of migration or intimal replication may go on at a low but steady rate.

Adhesion molecules and intimal formation

Surprisingly little has been done to identify the matrix molecules involved in migration into the intima. Osteopontin is of special interest because it is characteristically expressed in sites where tissues are undergoing marked remodeling including the notochord and sites forming new smooth muscle layers, including the gut and the neointima formed after injury, and the normal intima that forms spontaneously in the ductus arteriosus before birth (9). In vitro, osteopontin is a potent chemotactic factor and antibodies to osteopontin inhibit migration into the intima after balloon angioplasty (10).

While cells can attach to osteopontin via other α_v integrins, $\alpha_v\beta_3$ appears to be the major migration promoting receptor in vitro (11). This may be true in vivo as well since antagonists directed at $\alpha_v\beta_3$ inhibit formation of the intima after balloon injury (12, 13). These experiments seem to explain the apparent ability of ReoPro, an antibody against all β_3 integrins, to inhibit restenosis. However, caution needs to be expressed because the human studies lack evidence that intimal formation, rather than remodeling of the media, is the critical event in narrowing (1). Moreover, recent immunocytochemical studies from our laboratory found that $\alpha_v\beta_3$ was largely confined to the media rather than the intima where, one assumes, neointimal hyperplasia would have its pathologic effect (11).

It is important to point out that very little is known about other integrins present in vascular tissue. For example, while a number of in vitro studies have emphasized the role of $\alpha_2\beta_1$ integrins in interaction of cultured SMC with collagen, this integrin is not seen in vascular SMC in vivo. Instead, the major β_1 integrin seen in the intima in vivo is $\alpha_1\beta_1$. A recent paper suggests that this may be the major integrin involved in smooth muscle migration on collagen (14). On the other hand, while we know very little about the expression of integrins during vascular response to injury, a recent report on the intima of the ductus arteriosus reports that several integrins, including $\alpha_5\beta_1$ and $\alpha_v\beta_3$, are expressed during the rapid intimal formation seen in the ductus before birth (15). There is very little literature on the modulation of expression of integrins in other examples of intimal formation.

Proteoglycans may also be relevant to formation of the intima. CD44, a receptor for hyaluronic acid, has also been shown to play a role in migration of cells into fibrin or osteopontin (16, 17). CD44 is upregulated, along with hyaluronic acid in the neointima (18). Finally, the presence of hyaluronic acid in the intima may be very important itself. Hyaluronic acid accumulation is characteristic of the formation of a spontaneous neointima in the ductus arteriosus where it is believed to play a critical role in this process. In addition to CD44, migrating SMC express another hyaluronic receptor, RHAMM. Chemotaxis of cultured SMC is inhibited by HA-binding peptides or high doses of HA and high concentrations of HA in vivo inhibit neointimal formation.

The third class of relevant molecules are the matrix proteases. The cells forming the neointima have been shown to express several proteases including tissue type plasminogen activator, plasmin, MMP-2, and MMP-9 (19). The significance of these proteases is supported by three kinds of evidence. First, heparin, known to be a potent inhibitor of neointimal formation, inhibits induction of expression of tissue-type plasminogen activator as well as collagenase. This may be due to the ability of heparin to wash free FGF out of the wall as FGF has also been shown to induce transcription of plasminogen activator. Similarly, PDGF induces expression of a number of proteases and, as already noted, anti-PDGF inhibits smooth muscle migration. Finally, protease inhibitors inhibit intimal formation (19).

Other morphogenic processes in vascular pathology

While this review has emphasized intimal formation after injury, clonality suggests that plaques arise by clonal expansion of cells already in the intima. We do not know how the normal intima forms. For example clonality could be the result of trapping of rare cells within the forming internal elastic lamina (1). In summary, we do not know if the intimal hyperplasia is a key event in atherosclerosis or restenosis.

However, it is likely that other morphogenic processes are involved in atherosclerosis. For example, vascular narrowing may well depend on some form of smooth muscle migration. The size of atherosclerotic plaques does not correlate with the extent of vascular narrowing (20). As shown in Fig. 1, it is possible that repeated episodes of plaque rupture create wounds, and vessels narrow because of wound contracture. Indeed, intravascular ultrasound studies of vessels after angioplasty show just such wounds and the narrowing called "restenosis" seems to occur, in most cases, without addition of mass to the vessel wall (21). Unfortunately, there is no literature on the mechanisms controlling wound contracture in vessel walls but we assume that SMC migration would be critical to this process. The other possibility shown in Fig. 1 is that narrowing is the result of failure of a remodeling mechanism that permits expansion of the lumen as the lesion grows. Even less is known about how such a process might occur. However, a recent paper suggests an intriguing possibility. Mogford et al. showed that $\alpha_v\beta_3$ may regulate contraction of the vessel wall (22). Thus, integrins may play a role in active contraction as well as migration of SMC, providing a possible unifying mechanism for vascular remodeling.

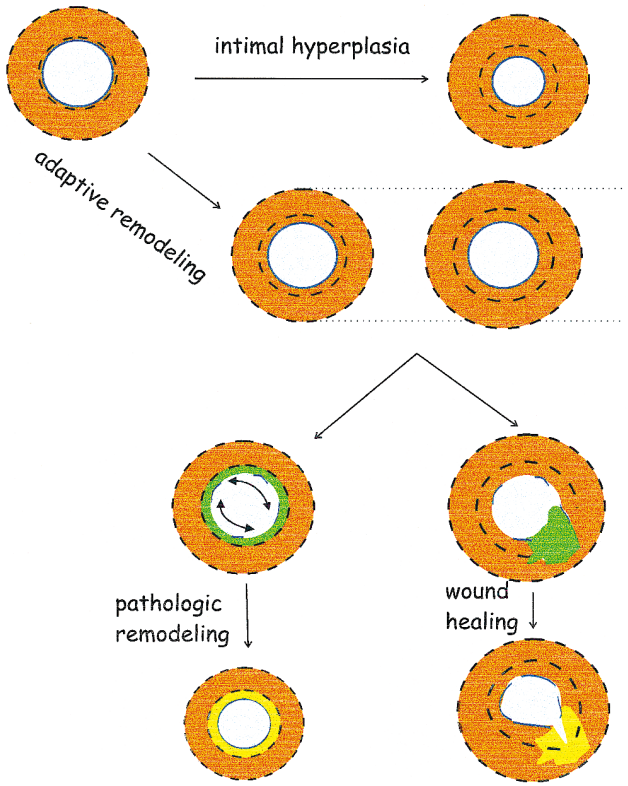

Figure 1.

Clinical relevance

Antimigratory drugs directed at formation of the intima may be of little interest if intimal formation is an early developmental event. Indeed, there is some reason to believe that resistance to plaque rupture may be dependent on formation of a fibrous cap so any therapy directed at inhibiting intimal formation may be a bad idea. However, narrowing is a very intriguing clinical target, one where the processes and relevant molecules need to be defined. Especially in the case of stent stenosis and transplant rejection, this may depend on intimal formation. On the other hand, in atherosclerotic progression and postangioplasty restenosis remodeling and wound healing may be the appropriate targets.

References

1. Schwartz, S.M., D. deBlois, and E.R.M. O'Brien. 1995. The intima: soil for atherosclerosis and restenosis. *Circ. Res.* 77:445–465.

2. Majesky, M.W., and S.M. Schwartz. 1997. An origin for smooth muscle cells from endothelium? *Circ. Res.* 80:601–603.

3. Lowery, M.C., J.C. Carey, M. Keating, and A.R. Brothman. 1995. Strong correlation of elastin deletions, detected by FISH, with Williams syndrome: evaluation of 235 patients. *Am. J. Hum. Genet.* 57:49–53.

4. Bostrom, H., K. Willetts, M. Pekny, P. Leveen, P. Lindahl, H. Hedstrand, M. Pekna, M. Hellstrom, S. Gebre Medhin, M. Schalling, et al. 1996. PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. *Cell.* 85:863–873.

5. Murry, C.E., C.T. Gipaya, T. Bartosek, E.P. Benditt, and S.M. Schwartz. 1997. Monoclonality of smooth muscle cells in human atherosclerosis. *Am. J. Pathol.* In press.

6. deRuiter, M.C., R.E. Poelmann, J.C. VanMunsteren, V. Mironov, R.R. Markwald, and A.C. Gittenberger-de Groot. 1997. Embryonic endothelial cells transdifferentiate into mesenchymal cells expressing smooth muscle actins *in vivo* and *in vitro*. *Circ. Res.* 80:444–451.

7. Li, J., L. Sensebé, P. Hervé, and P. Charbord. 1995. Nontransformed colony-derived stromal cell lines from normal human marrows. II. Phenotypic characterization and differentiation pathway. *Exp. Hematol.* 23:133–141.

8. Bennett, M.R., V. Lindner, D. deBlois, M.A. Reidy, and S.M. Schwartz. 1997. The effect of antisense C-MYC oligonucleotides on the rat carotid artery: dissecting the mechanism of action. *Arterioscler. Thromb.* In press.

9. Thayer, J.M., C.M. Giachelli, P.E. Mirkes, and S.M. Schwartz. 1995. Expression of osteopontin in the head process late in gastrulation in the rat. *J. Exp. Zool.* 272:240–244.

10. Liaw, L., D.M. Lombardi, M.M. Almeida, S.M. Schwartz, D. deBlois, and C.M. Giachelli. 1997. Neutralizing antibodies directed against osteopontin inhibit rat carotid neointimal thickening following endothelial denudation. *Arterioscler. Thromb. Vasc. Biol.* 17:188–193.

11. Hoshiga, M., C.E. Alpers, L.L. Smith, C.M. Giachelli, and S.M. Schwartz. 1995. avb3 integrin expression in normal and atherosclerotic artery. *Circ. Res.* 77:1129–1135.

12. Matsuno, H., J.M. Stassen, J. Vermylen, and H. Deckmyn. 1994. Inhibition of integrin function by a cyclic RGD-containing peptide prevents neointima formation. *Circulation.* 90:2203–2206.

13. Choi, E.T., L. Engel, A.D. Callow, S. Sun, J. Trachtenberg, S. Santoro, and U.S. Ryan. 1994. Inhibition of neointimal hyperplasia by blocking a_vb_3 integrin with a small peptide antagonist *GpenGRGDSPCA*. *J. Vasc. Surg.* 19:125–134.

14. Gotwals, P.J., G. Chi Rosso, V. Lindner, J. Yang, L. Ling, S.E. Fawell, and V.E. Koteliansky. 1996. The $\alpha 1\beta 1$ integrin is expressed during neointima formation in rat arteries and mediates collagen matrix reorganization. *J. Clin. Invest.* 97:2469–2477.

15. Clyman, R.I., B.W. Goetzman, Y.Q. Chen, F. Mauray, R.H. Kramer, R. Pytela, and L.M. Schnapp. 1996. Changes in endothelial cell and smooth muscle cell integrin expression during closure of the ductus arteriosus: an immunohistochemical comparison of the fetal, preterm newborn, and full-term newborn rhesus monkey ductus. *Pediatr. Res.* 40:198–208.

16. Svee, K., J. White, P. Vaillant, J. Jessurun, U. Roongta, M. Krumwiede, D. Johnson, and C. Henke. 1996. Acute lung injury fibroblast migration and invasion of a fibrin matrix is mediated by CD44. *J. Clin. Invest.* 98:1713–1727.

17. Weber, G.F., S. Ashkar, M.J. Glimcher, and H. Cantor. 1996. Receptor-ligand interaction between CD44 and osteopontin (Eta-1). *Science (Wash. DC).* 271:509–512.

18. Jain, M., Q. He, W.S. Lee, S. Kashiki, L.C. Foster, J.C. Tsai, M.E. Lee, and E. Haber. 1996. Role of CD44 in the reaction of vascular smooth muscle cells to arterial wall injury. *J. Clin. Invest.* 97:596–603.

19. Bendeck, M.P., C. Irvin, and M.A. Reidy. 1996. Inhibition of matrix metalloproteinase activity inhibits smooth muscle cell migration but not neointimal thickening after arterial injury. *Circ. Res.* 78:38–43.

20. Clarkson, T.B., R.W. Prichard, T.M. Morgan, G.S. Petrick, and K.P. Klein. 1994. Remodeling of coronary arteries in human and nonhuman primates. *J. Am. Med. Assoc.* 271:289–294.

21. Mintz, G.S., J.A. Kovach, S.P. Javier, C.J. Ditrano, and M.B. Leon. 1993. Geometric remodeling is the predominant mechanism of late lumen loss after coronary angioplasty (abstract). *Circulation.* 88 (No. 4, Pt. 2):I-654.

22. Mogford, J.E., G.E. Davis, S.H. Platts, and G.A. Meininger. 1996. Vascular smooth muscle alpha(v)beta(3) integrin mediates arteriolar vasodilation in response to RGD peptides. *Circ. Res.* 79:821–826.

"Cell Adhesion In Vascular Biology"

Series Editors, Mark H. Ginsberg, Zaverio M. Ruggeri, and Ajit P. Varki

October 15, 1996	Adhesion and signaling in vascular cell–cell interactions	Guy Zimmerman, Tom McIntyre, and Stephen Prescott
November 1, 1996	Endothelial adherens junctions: implications in the control of vascular permeability and angiogenesis	Elisabetta Dejana
November 15, 1996	Genetic manipulation of vascular adhesion molecules in mice	Richard O. Hynes and Denisa D. Wagner
December 1, 1996	The extracellular matrix as a cell cycle control element in atherosclerosis and restenosis	Richard K. Assoian and Eugene E. Marcantonio
December 15, 1996	Effects of fluid dynamic forces on vascular cell adhesion	Konstantinos Konstantopoulos and Larry V. McIntire
January 1, 1997	The biology of PECAM-1	Peter J. Newman
January 15, 1997	Selectin ligands: Will the real ones please stand up?	Ajit Varki
February 1, 1997	Cell adhesion and angiogenesis	Joyce Bischoff
February 15, 1997	von Willebrand Factor	Zaverio Ruggeri
March 1, 1997	Therapeutic inhibition of carbohydrate–protein interactions in vivo	John B. Lowe and Peter A. Ward
March 15, 1997	Integrins and vascular matrix assembly	Erkki Ruoslahti
April 1, 1997	Platelet GPIIb/IIIa antagonists: The first anti-integrin receptor therapeutics	Barry S. Coller
April 15, 1997	Biomechanical activation: An emerging paradigm in endothelial adhesion biology	Michael A. Gimbrone, Jr., Tobi Nagel, and James N. Topper
May 1, 1997	Heparan sulfate proteoglycans of the cardiovascular system. Specific structures emerge but how is synthesis regulated?	Robert D. Rosenberg, Nicholas W. Shworak, Jian Liu, John J. Schwartz, and Lijuan Zhang
May 15, 1997	New insights into integrin–ligand interaction	Robert Liddington and Joseph Loftus
June 1, 1997	Adhesive interactions of sickle erythrocytes with endothelium	Robert P. Hebbel
June 15, 1997	Smooth muscle migration in atherosclerosis and restenosis	Stephen M. Schwartz
July 1, 1997	Integrin signaling in vascular biology	Sanford Shattil and Mark Ginsberg
July 15, 1997	Multi-step mechanisms of leukocyte homing	Eugene Butcher
August 1, 1997	Role of PSGL-1 binding to selectins in leukocyte recruitment	Rodger McEver and Richard Cummings