ELSEVIER

Cardiovascular Research 41 (1999) 345–360

Cardiovascular Research

Review

Fibrous cap formation or destruction — the critical importance of vascular smooth muscle cell proliferation, migration and matrix formation

Andrew C. Newby*, Alla B. Zaltsman

Bristol Heart Institute, University of Bristol, Bristol Royal Infirmary, Bristol BS2 8HW, UK

Received 24 June 1998; accepted 19 August 1998

Abstract

Endothelial activation and infiltration of monocyte macrophages are essential prerequisites for fibrous cap formation, which comprises proliferation and migration of smooth muscle cells and net matrix deposition. Macrophage foam cells and endothelium act as a source of growth factors and chemoattractants for smooth muscle cells. However, growth factors alone do not stimulate smooth muscle cell proliferation or migration. This requires, in addition, the remodelling of the extracellular matrix, at least partly mediated by metalloproteinases. In particular, loss of basement membrane components and contact with the interstitial matrix appears to be required to release a brake on proliferation and migration exerted by the basement membrane. Unless there is a change in the phenotype of macrophages in advanced lesions, it is not clear why fibrous cap destruction rather than formation should take place in macrophage-rich shoulder regions of plaques. Impaired cap formation caused by smooth muscle senescence, mummification and propensity to apoptosis may be as important as increased cap destruction in promoting plaque rupture.

Keywords: Arteriosclerosis; Vascular smooth muscle; Extracellular matrix; Metalloproteinases

1. Introduction

The characteristic feature of the advanced atherosclerotic plaque is irregular thickening of the arterial intima by inflammatory cells, extracellular lipid (atheroma) and fibrous tissue (sclerosis) (Fig. 1). A large part of the lesions comprise seemingly inert and acellular fibrous tissue but there is often a distinct and highly cellular fibrous cap (Fig. 1). In eccentric lesions, such as the one shown in Fig. 1, there is also a leucocyte-rich 'shoulder region' at the limit between the plaque and the relatively normal tissue. The fibrous cap undoubtedly contributes something to luminal encroachment but its importance has recently been emphasised as a strong determinant of the likelihood of plaque rupture, which leads in turn to thrombosis followed either by occlusion or episodic plaque expansion [1–4]. Fibrous cap formation arises from the migration and proliferation of vascular smooth muscle cells and from matrix deposition [5]. The mechanisms underlying these processes have rightly therefore been the focus for an enormous body of molecular and cellular biology literature, itself the subject of authoritative reviews and regular updates [6–11]. The purpose of this article is to focus critically on the extent to which these processes might determine plaque stability.

2. Evolution of the fibrous cap

2.1. Experimental approaches

The primary tool for investigating the evolution of atherosclerosis in humans has been the careful observation of necropsy material from individuals of widely different ages [12,13]. The reasonable premise is that plaque age is somehow related to biological age. Alternatively, in fewer studies, the degree of stenosis has been taken as an indirect indicator of plaque age [14]. From observations of patients of different ages, fibrous cap formation appears to be a relatively late event in atherosclerosis, occurring after and, by implication, as an evolution from macrophage-rich fatty

*Corresponding author. Tel.: +44-117-928-3582; fax: +44-117-928-3581; email: a.newby@bris.ac.uk

Time for primary review 34 days.

0008-6363/99/$ – see front matter
PII: S0008-6363(98)00286-7

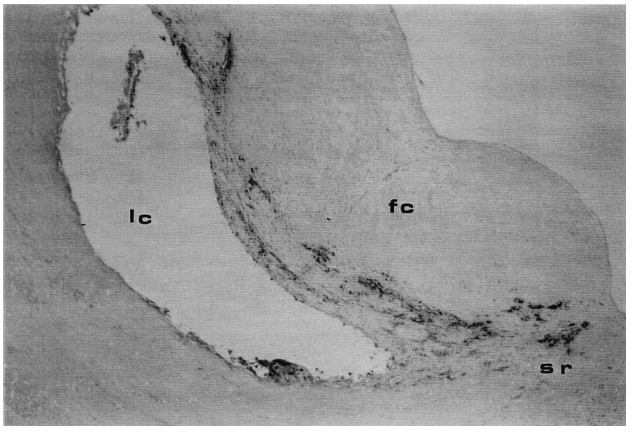

Fig. 1. Macrophage distribution in an advanced atherosclerotic plaque. A human carotid plaque was stained by immunocytochemistry of CD68 as a marker of macrophages (brown stain). fc=fibrous cap, lc=lipid core, sr=shoulder region. The scale bar shows 100 μm.

streak lesions [15]. This sequence of events is consistent with studies of cholesterol fed rabbits [16,17] and primates [18–21] and with observations on genetically hyperlipidaemic rabbits [22,23] and mice [24]. The animals used to model atherogenesis have a shorter life span than man and economic considerations preclude observations over the greater than 20 years that human lesions require to evolve into clinically apparent disease. The hope is that the evolution of lesions in these models mirrors that in man except in being conveniently accelerated. An additional difference between man and most of the animal models, except swine [25] and primates [20], is the presence of a significant population of mesenchymal cells, presumed to be vascular smooth muscle cells, in the normal arterial intima. This population and, hence, neointimal thickness is expanding throughout life, even in the absence of atherosclerosis. Studies by Smirnov's group (Orekhov et al. [26]) in which the intimal cell population in the aorta was counted showed that human aortic atherosclerotic plaques contained approximately twice as many smooth muscle cells as found in the neighboring normal areas. Pathological studies demonstrate that atherosclerotic plaques develop within preexisting areas of neointimal thickening in man [27], an observation confirmed in an experimental swine model [25].

2.2. Determinants of fibrous cap morphogenesis

Plaque morphology is generated by the organised motion of the constituent cell types, namely vascular smooth muscle cells and various blood leucocytes. As shown in Fig. 2a, the initial and most well established event is the movement of blood monocytes across the endothelium and their transformation to tissue macrophages in the subendothelial matrix [28–32]. Entry of further monocytes and lymphocytes leads to the fatty streak, which is regarded as a stable lesion, due perhaps to its low haemodynamic profile. Expression of endothelial leukocyte adhesion molecules and production of macrophage chemoattractants, including monocyte chemotactic peptide-1 (MCP-1), from smooth muscle cells [33] may orchestrate this motion. Oxidation of low-density lipoprotein (LDL) that has entered the vessel wall has a central role in stimulating adhesion molecule and MCP-1 expression [34]. Subsequently, there appears to be migration of smooth muscle from the media into the intima (Fig. 2a). This is clearly visible at early time points in both mice and rabbits (Fig. 3), where there is no pre-existing intimal population. In man and other animals where there is already an intimal smooth muscle cell population, it is possible that plaque smooth muscle cells derive solely from these cells [8]. Interestingly, smooth muscle cell proliferation has been observed in diffuse intimal thickenings [35], which implies that this population could expand to provide plaque smooth muscle cells. However, the frequent observation of large disruptions of the internal elastic lamina and wasting of the media at the base of human atherosclerotic plaques (see, for example, reference [36]) strongly suggests that at least some plaque smooth muscle cells are recruited from the media.

Data from human plaques of type III, before formation of a distinct fibrous cap [15], apoE-deficient mice on a Western-type diet [24] and cholesterol-fed rabbit aortas (e.g. Fig. 3) suggest that migration of smooth muscle cells then occurs through foam cell-rich areas, as illustrated schematically in Fig. 2b. Production of platelet-derived growth factor (PDGF) B chains from macrophages, which has been observed at all stages of atherogenesis in both human and experimental models [5,37,38], is probably relevant in recruiting smooth muscle cells to the intima. Heparin-binding epidermal growth-factor-like growth factor (HB-EGF) from macrophages also plays a role in smooth muscle cell (SMC) migration [39]. However, the expected outcome would be a mosaic of macrophages and smooth muscle cells, as seen in Fig. 3. Additional mechanisms must explain the formation of a distinct fibrous cap. Sorting of cells through cell surface cadherins may contribute [40], since smooth muscle cells, but not macrophages, express N-cadherin [41]. Homotypic binding between arrays of N-cadherin monomers could then lead to strong smooth muscle cell-to-cell interactions that would exclude macrophages. N-cadherin is also expressed together with VE-cadherin on endothelial cells [41] and this favours endothelial cell smooth muscle cell contacts [42].

Migration of smooth muscle cells along an endothelial to medial gradient of chemoattractant is another likely mechanism underlying cap formation (Fig. 2c). Activated endothelium [43] produces PDGFBB [44] and insulin-like growth factor-1 (IGF-1) [9]. The endothelium is essential for neointimal migration of smooth muscle cells in organ cultures of pig aorta [45]. The presence of endothelium [46] and PDGF [47] are also both necessary for neointimal migration of smooth muscle cells in human saphenous

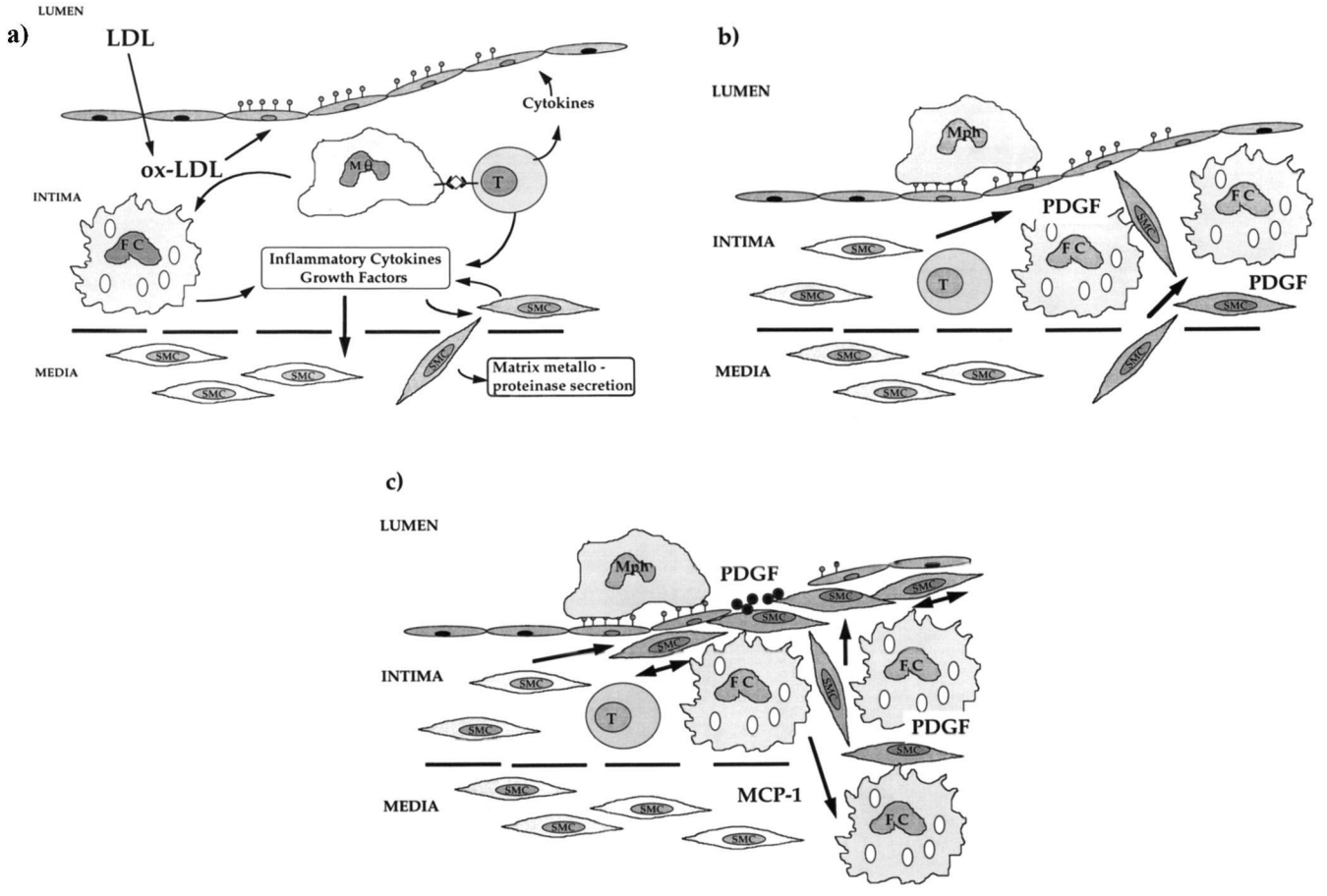

Fig. 2. Scheme of cell movements in plaque morphogenesis. (a) LDL and monocytes move through the endothelium, where oxidised LDL is formed, stimulates production of MCP-1 and is taken up to generate macrophage-derived foam cells. (b) Macrophage-derived PDGF and other factors (not shown) encourage medial and pre-existing intimal SMC to migrate through and around foam cells. (c) Macrophage, endothelial and platelet-derived PDGF promote further migration and proliferation of SMCs to produce a distinct fibrous cap. Macrophages penetrate into the media attracted by MCP-1. FC=foam cell, MCP-1=monocyte chemotactic peptide-1, Mph=monocyte/macrophage, (ox)-LDL=(oxidised) low density lipoprotein, PDGF=platelet-derived growth factor, SMC=smooth muscle cell, T=T lymphocyte.

veins. A further possibility is PDGFAB production from platelets, where there is evidence for platelet adhesion to sub-endothelium in the late stage of atherogenesis (Fig. 2c) [21,48]. An additional possibility is fibrin degradation products, which are known to arise from plasma fibrinogen and to adopt a subendothelial to medial concentration gradient in human plaques [49,50]. Perhaps these factors act in concert.

Apart from the medial to intimal migration of smooth muscle cells described in Fig. 2, there is also circumferential motion, leading to expansion of the media. This accounts for the well accepted compensatory increase in arterial diameter with atherosclerosis [51], and for the initial increase in lumen diameter in vein grafts [52].

A further event may be penetration of macrophages into the media (Fig. 2c), for which there is good evidence in mouse [53] and Watanabe rabbit models [54]. In human coronary arteries and, particularly in aneurysmal dilatation of the abdominal aorta, there is wasting and inflammatory infiltration of the media, which may be analogous to the situation in $ApoE^{-/-}$ mice [53,55]. Cholesterol-fed, genetically normal rabbits seem to have more superficially superimposed lesions (Fig. 3). Similarly, in human coronary vein grafts, lesions often show little penetration into the previously thickened neointima, which leads to the invidious, friable toothpaste-like lesions that are characteristic of failed grafts [56]. In the later stages of fibrous plaque morphogenesis (Fig. 2c), there may be migration of smooth muscle cells away from sites of cell proliferation. At the same time, penetration of fresh monocytes must be preferentially directed to the perimeter of the fibrous cap to explain the existence of leucocyte-rich shoulder regions. Mast cells are detected preferentially in the shoulder regions of later stage atheromas [57]. These might contribute to the fresh entry of macrophages and lymphocytes as well as to T lymphocytes growth by producing tumour necrosis factor α (TNFα) [58]. TNFα is a mediator of endothelium–leukocyte adhesion molecule-1 induction [59] and an enhancer of T lymphocyte growth [60]. Macrophages and endothelial-derived chemoattractants in

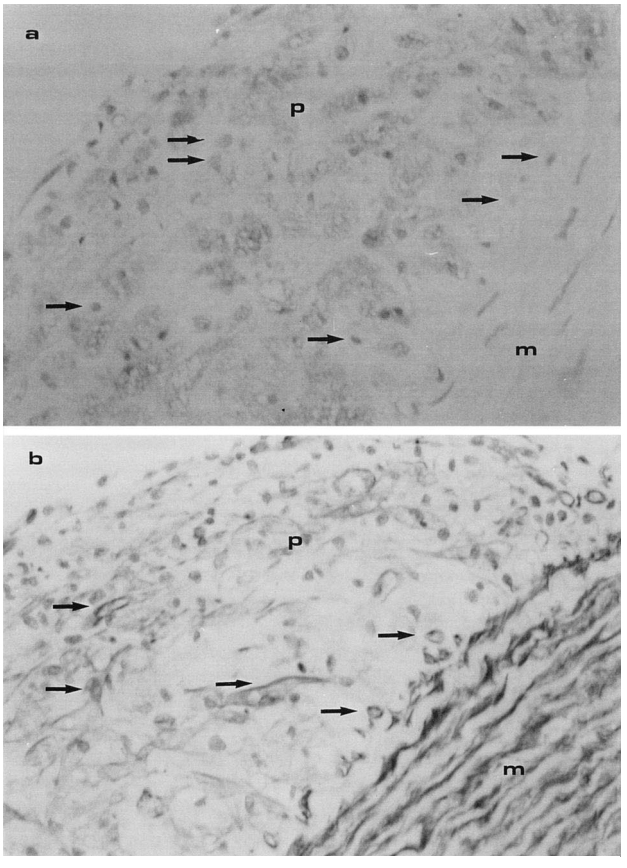

Fig. 3. Smooth muscle and macrophage distribution in an early aortic atherosclerotic plaque from a cholesterol-fed rabbit. Rabbits were fed a 1% cholesterol diet for eight weeks [149]. Sections were stained for (A) macrophages (RAM-11 antibody) and (B) vascular smooth muscle cells (α-smooth muscle actin) [149]. Arrows denote the presence of smooth muscle cells both at the margin between the media (m) and plaque (p) and surrounded by macrophages. The scale bar represents 25 μm.

these shoulder regions presumably attract further smooth muscle cells either from the subjacent media or from the edges of the existing fibrous cap.

2.3. What is the source of the neointimal connective tissue?

A pervasive concept, advanced by Chamley-Campbell and Campbell [61], is that normal contractile smooth muscle cells can undergo reversible modulation into a synthetic phenotype that is capable of both proliferation and enhanced matrix synthesis [62]. The response to vascular injury, such as balloon dilatation, clearly demonstrates that smooth muscle cells, as a population, possess the ability to change phenotype [63,64]. However, cell proliferation can occur before and, hence, independently of phenotypic modulation [65,66]. What remains uncertain is whether or not the sluggish proliferation in atherosclerotic plaques results from phenotypic modulation of typical contractile smooth muscle cells. Data from several species suggests the possibility that the smooth muscle cells in arteries represent several phenotypes in a lineage. Schwartz et al. [7] and Clowes and Schwartz [63] originally observed that adult rat arteries retain a population of smooth muscle cells with a neonatal epithelioid phenotype. Moreover, intimal smooth muscle cells share many aspects of this phenotype, suggesting that they may be derived selectively from it [67]. The epithelioid phenotype has not yet been identified in other species, including man. However, bovine pulmonary arteries contain four distinct cell phenotypes, at least two of which are clearly smooth muscle cells [68]. Observations of cell shape in alcoholic–alkaline etched preparations of human aorta also identify four populations (elongated, elongated with side process, irregular and stellate). The proportion of the stellate phenotype, possibly equivalent to the senescent phenotype in culture, increases in atherosclerotic tissue [12,26] or in vitro in response to transforming growth factor-β (TGF-β) [69].

Some recent observations may cast doubt on whether the atherosclerotic neointimal mesenchymal cells derive from differentiated smooth muscle cells at all. Many lesional neointimal cells stain poorly for typical markers of smooth muscle cells, including desmin and smooth muscle cell α-actin or myosin [70–72]. Some authors suggest a fibroblast origin for these cells [73], while others suggest, more cautiously, an indeterminate origin [66,68,74]. Adventitial fibroblasts expressing smooth muscle cell α-actin have been identified migrating into the thickened neointima in experimental venous arterial interposition grafts [75] and in restenotic lesions after angioplasty [73]. Development of more selective immunocytochemical markers that differentiate between smooth muscle cells and fibroblasts is necessary to determine if, and to what extent, plaque caps may be derived from myofibroblasts.

2.4. The role of cell proliferation

Simplistically, the data from Smirnov's studies, alluded to above, imply that a single population doubling in 20 years, equivalent to a proliferative index of approximately 0.01%, is sufficient to account for the increase in smooth muscle cell numbers in atherosclerotic plaques. The large amounts of acellular connective tissue in plaques [14] may imply the net loss of a further one or two cell doublings through necrosis or apoptosis. Even so, the very low rates of cell proliferation that have been observed in plaques (0–1% PCNA proliferation index) [35] are entirely consistent with the extent of intimal enlargement. These very low rates cannot be used therefore to discount the importance of proliferation as a component of atherogenesis. Smooth muscle cells are capable of doubling within 48 h in tissue culture or immediately after balloon injury [65]. The most interesting question is therefore not so much why smooth muscle cells proliferate in atherosclerosis but how their proliferation is so effectively suppressed in the normal vessel wall.

A parameter more difficult to define is the number of population doublings undergone by individual intimal cells. Benditt and Benditt [76] were the first to observe that lesional smooth muscle cells appear in many cases to have derived by clonal expansion of a small number of cells (or perhaps just one). Although not strictly proven, more modern polymerase chain reaction (PCR)-based methods [77] have largely confirmed the early work. At the same time, lesion smooth muscle cells display properties of transformed cells; their DNA transforms cultured fibroblasts that produce tumours in nude mice [78,79]. Furthermore, plaque smooth muscle cells, like many transformed cells, show greater susceptibility to apoptosis [80]. If all of the intimal smooth muscle cells in a typical coronary atherosclerotic plaque, perhaps 10^5 [26], were derived over 20 years from a single cell, this represents only one doubling per year, an index of proliferation of 0.1%, which is still consistent with experimentally measured values. As a result, such cells might be close to their Hayflick limit [81] and, hence, tending towards senescence, for which there is morphological evidence, as detailed above.

2.5. What regulates smooth muscle cell proliferation in atherogenesis?

We previously proposed objective criteria, which are updated and generalised in Table 1, to evaluate the role of individual factors in aspects of atherogenesis [82]. Growth factors for isolated human smooth muscle cells can be categorised according to the structure of their receptors. PDGF, basic and acidic fibroblast growth factors (bFGF, aFGF), IGF-1 and epidermal growth factor (EGF) all have receptors with intrinsic tyrosine kinase activity [9,83]; angiotensin II, endothelin, 5-hydroxytryptamine and thrombin have serpentine G-protein-coupled receptors [82,84,85], TGF-β has receptors with intrinsic serine/threonine kinase activity [86] and interleukin-1 (IL-1) and TNF-α have receptors that couple indirectly to serine/threonine kinases [87]. All of these agents fit criterion 1, having been shown to promote smooth muscle cell proliferation (see [7,82] for reviews) either directly, or indirectly by upregulating production of another growth factor or its receptor. TGF-β has biphasic stimulatory and inhibitory effects on proliferation, mediated respectively by type I and II receptors [86,88]. Given their importance in atherogenesis, the role of normal and oxidised lipoproteins in regulating smooth muscle cell proliferation has been extensively studied (see reference [89]). Several normal lipoprotein fractions stimulate smooth muscle cell proliferation either by acting as a nutrient [90] or a specific mitogen [89,91]. Oxidised lipoproteins generally cause a decrease in smooth muscle cell expansion rates but through promoting apoptosis [91].

Criterion 2, that the factor and its receptors are present in plaques at sufficient levels, has been met by fewer agents. One problem is that atherogenesis is both slow and episodic and, hence, the factor may only be present transiently or patchily. The concentration of PDGF A and B chains is increased in atherosclerotic tissue [5,92,93], although recent data demonstrated more PDGFA in normal arteries than in plaques [94]. PDGFB chain protein has been associated, by immunocytochemistry, with macrophages and by in situ hybridisation with neointimal mesenchymal cells and endothelial cells in human atherosclerotic plaques [71]. PDGF α and β receptors are also abundant on smooth muscle cells in human atherosclerotic lesions [37,71,95]. The concentrations of IGF-1 [96] and TGF-β [97] are also elevated in primary atherosclerotic and restenotic tissues. The type II TGF-β receptors, associated with inhibition of proliferation, appear to be downregulated in neointimal smooth muscle cells, perhaps leading to unmasking of proliferative action through the type I receptor or through other factors [88]. Acidic and basic FGF are both present in normal and plaque smooth muscle cells and endothelium, although mainly sequestered in cells or bound to the extracellular matrix [98,99]. Hence, their availability at FGF receptors, other than as a result of injury and cell death, is uncertain. Thrombin activity is increased in neointima and thrombin receptors are overexpressed in human atherosclerotic plaques [100–102].

With the possible exception of endothelin-1 [103], no growth factor has so far met criterion 3, that an inhibitor should prevent atherosclerosis. In the balloon-injured rat carotid artery, anti-PDGF antibodies inhibit migration of smooth muscle cells into the intima [104] while anti-bFGF antibodies inhibit the early proliferative response but do not reduce final intimal thickness [105]. A combination of anti-PDGF and anti-bFGF antibodies greatly inhibits neointima formation [106]. Antibodies to TGF-β demonstrate inhibitory activity against neointima formation in the same rat model [107]. Angiotensin-converting enzyme inhibitors or angiotensin receptor-1 blockers and endothelin receptor antagonists also partly inhibit neointima formation [108,109]. Antithrombin agents also have inhibitory effects on neointima formation [110].

From these studies, multiple growth factors appear to act together to stimulate neointima formation in balloon-injured arteries by cooperative and, in cases, synergistic

Table 1
Criteria to assess the involvement of factors in atherogenesis

Criterion 1	The factor must act on isolated cells and when infused or transfected into animals.
Criterion 2	The factor and its receptor must both be present and shown to be active at some stage of atherogenesis.
Criterion 3	Selective inhibitors or gene deletion studies should reverse an aspect of atherosclerosis.

interactions. To what extent these conclusions can be extrapolated to atherosclerosis formation remains uncertain, however. Recent experience with expensive and unsuccessful clinical trials of many inhibitors of smooth muscle cell proliferation in angioplasty restenosis graphically illustrate the potential pitfalls of such premature extrapolation [8].

2.6. Growth factors are not enough to stimulate smooth muscle cell proliferation

The mitogenic action of growth factors on smooth muscle cells is dramatically modified by their microenvironment within the vessel wall. This is shown clearly in organ culture experiments of rabbit and pig aorta and of human saphenous veins (see Fig. 4). Exposure to serum, which is mitogenic for the same vesicular smooth muscle cells (VSMCs) when isolated, either fails entirely to stimulate VSMC proliferation [111] or does so selectively in the subendothelial space (Fig. 4a, Fig. 4c) [45,46]. Proliferation in the media occurs only when this is injured (Fig. 4c) [111,112]. These observations have counterparts in the balloon-injured rat carotid model where medial proliferation and the extent of neointima thickening depends not primarily on the extent of endothelial injury and, hence, exposure to platelet-derived mitogens but on the degree of medial injury [113]. One possible explanation for these phenomena is that multiple growth factors (e.g. PDGF plus bFGF) need to be simultaneously released by a variety of stimuli [105], or additional factors (e.g. proteases) are needed to achieve access of growth factors to their receptors [114]. Alternatively, we have proposed that interactions with the extracellular matrix surrounding smooth muscle cells in the normal artery strongly suppress proliferative responses [115]. This hypothesis depends in part on data from Clowes' and Campbell's groups, demonstrating, in a series of classic experiments, the inhibitory effects of heparin on smooth muscle proliferation [116,117] The same effect is obtained with heparan sulphate proteoglycans, many of which (e.g. syndecans, perlecan) are prominent components of the basement membrane that normally surrounds smooth muscle cells. Heparin and, hence, presumably heparan sulphates act directly against proliferation by inhibiting the binding of the activator protein-1 (c-fos/c-jun) transcription factor to its binding sites in DNA [118]. This mechanism probably accounts also for the inhibitory effects of heparin on secretion of extracellular proteases including tissue plasminogen activator (t-PA) and matrix metalloproteinases (MMPs) [118–121]. Work by Hedin et al. [122,123] and Thyberg and Hedin [124] also shows that the basement membrane component, laminin, inhibits while the interstitial matrix component, fibronectin, promotes phenotypic modulation of smooth muscle cells. Based on such evidence, we have developed a detailed hypothesis, as illustrated in Fig. 5a, proposing that the basement membrane

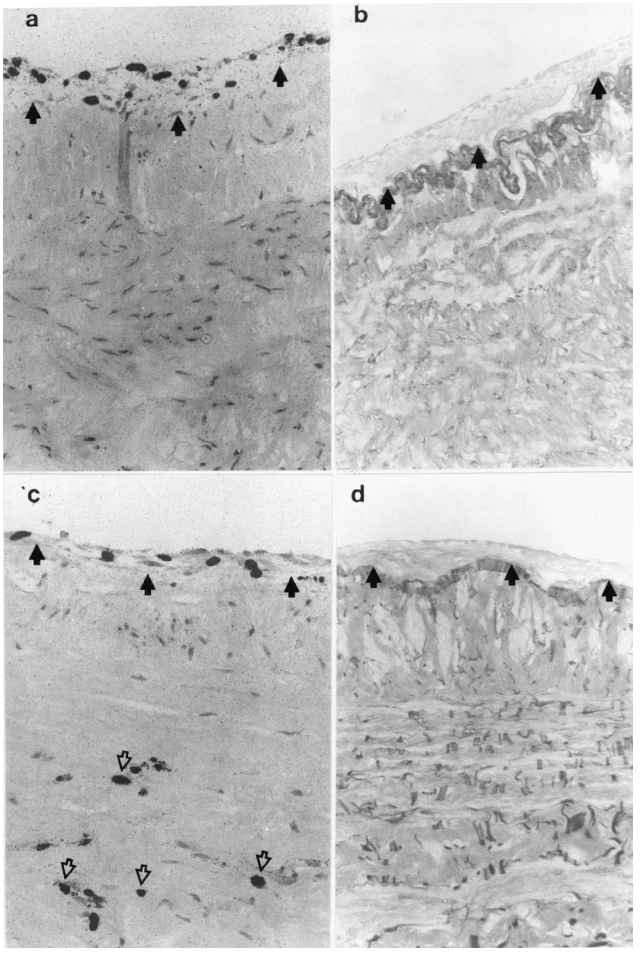

Fig. 4. Cell proliferation in organ cultures of human saphenous vein. (a) A segment of undamaged 'freshly isolated' vein cultured in 30% serum and 1 μCi/ml of [^{3}H]thymidine for 14 days. The section has been stained with haematoxylin and eosin and subjected to autoradiography to reveal the presence of cells that have undergone DNA synthesis. In this section, dividing cells are restricted to the neointima (above the arrows). (b) A serial section to (a), stained to reveal the internal elastic lamina (arrows). (c) A segment of damaged 'surgically prepared vein' treated as in (a). Note the presence of dividing cells in the media (below the arrows). (d) A serial section to (c), revealing the internal elastic lamina (arrows). The scale bar represents 25 μm. The figure is adapted from reference [112].

provides a 'brake' on proliferation of smooth muscle cells [11,83,115,125]. Important support for this hypothesis has come from Ross's group, who showed that defined extracellular matrix components can prevent smooth muscle cells from passing through the cell cycle by upregulating the p^{27} cyclin-dependent kinase inhibitor [126]. Furthermore, we proposed that extracellular proteolysis is the rate-limiting step in catabolism of all basement membrane components and is, hence, the regulated step in relieving the inhibitory constraints on proliferation [83]. The basement membrane-degrading metalloproteinase, MMP-2, is produced constitutively by smooth muscle cells [115,127] and is activated either by membrane-type metalloproteinases [128], thrombin [129] or reactive oxygen species [130]. A second basement membrane-degrading metallo-

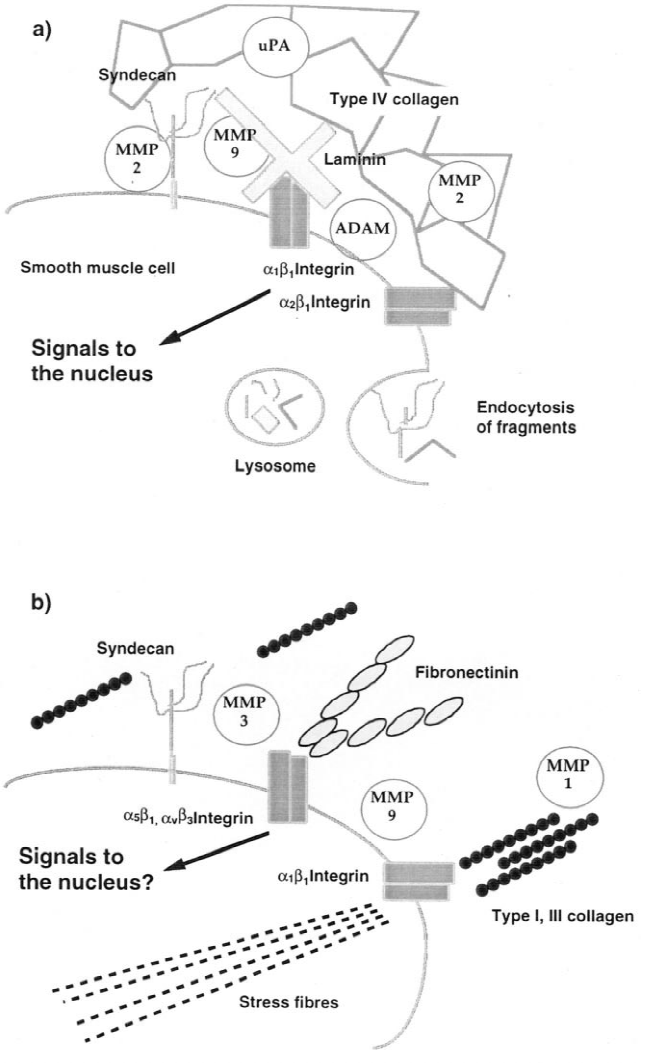

Fig. 5. Matrix interactions in contractile and synthetic smooth muscle cells — the 'brake' hypothesis. (a) Contractile smooth muscle cells are surrounded with a basement membrane of type IV collagen and laminin that is rich in heparin sulphate proteoglycans, including syndecans. Binding to β1 integrins generates a signal that maintains the contractile status and puts a brake on migration and proliferation. Release of the brake requires turnover of matrix components that is initiated by extracellular proteases including metalloproteinases (MMP), urokinase plasminogen activator (u-PA), and disintegrin and metalloproteinase-containing proteins (ADAMs). Endocytosis followed by further proteolysis and glycolysis completes the breakdown of matrix components. (b) Synthetic smooth muscle cells have degraded their basement membrane and come into contact with interstitial matrix components, including monomeric and polymerised types I and II collagen and fibronectin. These also bind to integrin, including, importantly, $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins, which may provide the signals for phenotypic change. Integrins can also act as points for organisation of stress fibres, while fibronectin and other glycoproteins provide track ways for migration. Activation of smooth muscle cells is associated with upregulation of MMPs that can promote turnover of the interstitial matrix.

proteinase, MMP-9, is induced by inflammatory cytokines [127], especially in combination with peptide growth factors [131].

Activation of MMP-2 and induction of MMP-9 are early events in the vascular response to injury [132–134]. Experiments in the human organ culture model demonstrated a spatial and temporal correlation between the expression of MMP-9 and medial cell proliferation in response to injury [135]. High concentrations of synthetic MMP inhibitors have been shown to inhibit proliferation of smooth muscle cells, particularly when proliferation is measured within the intact tissue in vitro [115] or at early time points after vascular injury in vivo [136]. On the other hand, other studies with synthetic inhibitors [137] or gene transfer of TIMP-1 in vivo [138] or by adenovirus-mediated delivery to human saphenous vein [139] showed no effect on proliferation. Hence, the role of basement membrane-degrading MMPs in regulating smooth muscle cell proliferation is uncertain. Heparanases [140,141] and other classes of proteases, for example plasmin, cathepsins [142] or the more recently described disintegrin and metalloproteinase-containing proteins (ADAMs) [143], might also have a role in regulating basement membrane integrity and cell proliferation.

In the context of atherosclerosis, there is strong evidence for the upregulation of interstitial stromelysin (MMP-3), collagenase (MMP-1), metalloelastase (MMP-12) and MMP-9 expression in human atherosclerotic plaques, in the cholesterol-fed rabbit and in genetically modified mice (criterion 2) [144–150]. Activity of these proteases, in particular MMP-2, is also increased [146,149]. Increased expression has been correlated with morphological and mechanical determinants of plaque rupture [144,145,147,148]. Indeed, a strong case has been made that macrophage-derived MMPs are a primary cause of plaque rupture [144,145,147,148]. However, in the rabbit and mouse models, increased MMP expression and activity occurs early in the development of lesions [53,146,149], implying that they have a role in plaque morphogenesis (i.e. cap formation) as well as plaque rupture. As yet, no protease has been shown by inhibitor studies to be essential for plaque formation or rupture (criterion 3), although the existence of knockout mice for many of these proteases promises much for the near future.

2.7. What regulates smooth muscle cell migration?

As with proliferation, directed smooth muscle migration requires the combined action of chemoattractants and remodelling of the extracellular matrix [151]. Matrix remodelling may take two forms, namely removal of constraints to movement and the formation of productive cell matrix interactions that are necessary for movement [9]. This in turn probably requires both activation of cell surface receptors for matrix molecules, including integrins, and the reorganisation of the matrix itself. Some of the important changes are illustrated in Fig. 5. An interesting unresolved question is whether the basement membrane simply acts as a physical barrier to movement, 'the cage

hypothesis', or whether signalling events actively suppress movement, 'the brake hypothesis' (Fig. 5).

Migration is frequently measured with recently dispersed cells crossing a microporous membrane (Boyden chamber assay) so as to minimise the effects of matrix interactions. Alternatively, reconstituted basement or interstitial matrix can be coated onto membranes in the same assay system so that chemotaxis and invasion can be measured together. When migration is studied from explants, in a complex organ culture or in an animal model, direct effects on chemotaxis and on matrix remodelling occur together, as they do physiologically. Not surprisingly, more regulatory interactions emerge the more complex the experimental model.

Using the Boyden chamber assay, some, but not all, smooth muscle mitogens have been shown to possess direct chemotactic activity, which implies that the pathways for stimulating migration are overlapping but distinct from those mediating proliferation. For example, IGF-1 is a weak activator of mitogenic pathways but potently induces migration of human VSMCs [152]. One of the most potent chemoattractants for smooth muscle cells is PDGFBB [5,9,38,153]. PDGFBB and PDGFAA are equally good activators of the intracellular mitogenic pathways, including the mitogen-activated protein kinase (MAP kinase) cascade and early response gene (e.g. *c-fos*) induction. However, PDGFAA either has no effect or actually antagonises the chemotactic actions of PDGFBB chains [154,155]. Lakatta's group has provided persuasive evidence that Ca^{2+} mobilization and Ca^{2+}–calmodulin-activated kinase II, rather than MAP kinase, mediates chemotaxis [156,157]. Other mitogens, including bFGF [158] and HB-EGF have also been shown to promote migration. The effects of growth factors on migration may be direct, indirect or both. For example, PDGF-BB induces synthesis of thrombospondin, which potentiates chemoattraction by PDGF [159]. Moreover, PDGF and other mitogens, especially when combined with inflammatory mediators, increase expression of MMPs [131,160].

When invasion through reconstituted matrix is added to the chemotaxis assay, clear evidence for the involvement of extracellular proteases, including t-PA [161] and urokinase plasminogen activator (u-PA) [162] emerges. MMPs are also centrally involved. Synthetic MMP inhibitors or overexpression of tissue inhibitors of metalloproteinases-1, -2 or -3 inhibit migration of smooth muscle cells through basement membrane [136,138,163,164]. The involvement of both classes of protease may be explained either by independent effects on matrix components or because plasmin is an activator of MMPs-1, -3, -7, -9 and -12.

Using more complex organ culture models, the importance of PDGF acting in concert with MMPs is confirmed. Migration of SMCs out of baboon aortic depends on MMP expression, and this is mediated by endogenous PDGF and bFGF [165]. Both anti-PDGF antibodies [47] and adenovirus-mediated overexpression of TIMP-1 [139] also inhibit neointima formation in human saphenous vein organ cultures by a selective effect on cell migration.

In vivo experiments in balloon-injured rat carotid arteries also confirm the importance of growth factors working together with extracellular proteases in SMC migration. Expression of t-PA and u-PA increases after balloon injury [166] and tranexamic acid, an inhibitor of plasminogen activation, decreases rat SMC migration in this model [167]. Increased t-PA activity is partly mediated by PDGF [168]. Overexpression of plasminogen activator inhibitor-1 (PAI-1) results in suppression of arterial neointimal formation in PAI-1-deficient mice [169]. In the case of MMPs, both synthetic inhibitors [136,137] and seeding of cells transfected with TIMP-1 [138] inhibit migration of SMCs and either delay [136,137] or inhibit [138] neointima formation.

2.8. Deposition of extracellular matrix

Contractile smooth muscle cells have low levels of total messenger RNA, demonstrating their limited capacity for new protein synthesis. Not surprisingly, therefore, synthesis of extracellular matrix molecules by contractile cells is limited, although basement membrane proteoglycans, including syndecans and perlecan, may be an exception [119]. Phenotypic modulation, provoked by isolation of cells from the tissue by collagenase, leads to a profound alteration in both the pattern (see Fig. 5) and extent of matrix molecule expression [170,171]. Increased synthesis of hyaluronic acid is an early event that is generally associated with the production of an emergency matrix after tissue injury [172,173]. Basement membrane components, for example laminin, are downregulated [174], while synthesis of type 1 collagen, elastin and fibronectin is greatly increased. The components of this matrix resemble the normal interstitial matrix, however, the abundance of monomeric rather than polymerised collagen may be of key importance in allowing cells to proliferate [126]. Fibronectin may polymerise into fibres, providing trackways for migration [175,176]. Osteopontin also has a migratory effect on SMCs, which is mediated by complexing with the $\alpha_v\beta_3$ integrin [177]. Interactions of both β_1 and β_3 integrins with the extracellular matrix are important for directed cell migration [178] (see Fig. 5). Vitronectin is also known to promote migration [179,180]. Coincident with confluency, isolated smooth muscle cells begin to re-express basement membrane components, consistent with their proposed role in promoting quiescence [174].

Given the slow and focal nature of proliferation in atherosclerotic plaques, the concomitant changes in matrix turnover would be expected to be subtle. What one observes in an overall comparison of diseased and normal tissue is likely to be a distant and more permanent record of the immediate changes. Plaques are characterised by increased levels of collagens I and III, and elastin, as well as chondroitin sulphate- and dermatan sulphate-containing

proteoglycans [181], components of interstitial rather than basement membrane. At the same time, atherosclerotic plaques have decreased levels of heparan sulphates, known components of basement membrane and inhibitors of SMC proliferation [181–183]. Prominent areas of collagen synthesis have been demonstrated within the fibrous cap [184], where the collagen producing smooth muscle cell is not necessarily proliferating [185]. The factors likely to be responsible for increased collagen production include PDGF and TGF-β [171]. This implies that PDGF and TGF produced by macrophages upregulate collagen and fibronectin production from smooth muscle cells [186,187] and, hence, further promote fibrous cap formation.

Many of these changes can be seen also in animal models of balloon injury [171]. Other matrix molecules that are upregulated in these models include the fibril-associated type VIII collagen [188] and the multiadhesive glycoproteins, osteopontin [189,190] and thrombospondin [159,191]. RGD-containing peptides, which block binding to integrins, inhibit neointimal formation after balloon injury [192] or stent injury [193].

3. Role of the smooth muscle cell in plaque rupture

3.1. Contrasting hypotheses

The histological appearance of rupture-prone plaques is dealt with thoroughly elsewhere in this volume. Put simply, rupture occurs in plaques with a large lipid core, a thin cap and where there are abundant macrophages in relation to smooth muscle cells [194,195]. It is perhaps the prevailing view that rupture results from the destruction of an established fibrous cap, and that this is mediated by macrophages [32,196,197]. An alternative interpretation is that new areas of fatty streak at the edges of existing fibrous plaque simply fail to generate or have not yet generated an adequate plaque cap. Table 2 lists six different hypotheses that might account for plaque rupture. Each has merit but gives central importance to a different component of the plaque. Below we consider these hypotheses from the perspective of smooth muscle cell biology.

3.1.1. Smooth muscle senescence

The direct evidence that only a tiny minority (i.e. clones) of cells respond to mitogenic stimuli in the slowly evolving human atherosclerotic plaque is reviewed above. If plaques are derived by clonal expansion, senescence becomes a real possibility that is supported by both morphological measures and the behaviour of plaque smooth muscle cells in culture. The presence of mediators such as TGF-β in the plaque may hasten senescence. The true cause of plaque rupture may therefore be the exhaustion of the reparative power of smooth muscle cells. If so, then the appropriate treatment, surprisingly, might be locally to reinforce the stimuli for proliferation with a growth factor such as PDGF. In this sense, it may be analogous to the observation that endothelial regrowth over a large denuded area of rat aorta remains incomplete, implying a limit to the regenerative capacity of the endothelium. This can be overcome, nevertheless, by infusion of a mitogen, basic FGF [198]. The vigorous smooth muscle cell response to gross angioplasty injury, metal stent implantation or deposition of a mural thrombus further implies that the senescent phenotype can be rescued by a sufficiently large stimulus.

3.1.2. Mummification

The difficulty in provoking the typical smooth muscle cell into proliferation has been emphasised throughout this article, as has the importance of basement membrane as an inhibitor. It seems highly unlikely therefore that the smooth muscle cells surrounded by multiple layers of basement membrane frequently found in atherosclerotic plaques [13] could play any part in fibrous cap formation. The reasons for the generation of this phenotype are unclear, however, it might also be rescued by the right stimulus.

3.1.3. Breakdown in the chemotactic gradients that promote cap formation

Aberrant regulation of the production of endothelial derived mediators is one of the earliest events in the preclinical phase of atherosclerosis. Increased expression of leukocyte adhesion molecules is another marker of dysfunction [43]. Late on in atherogenesis, focal loss of endothelial cells occurs [21,48,194]. Given these changes, it is likely that formation of endothelium-derived PDGFB-chains and other chemoattractants for smooth muscle cells is impaired also.

Table 2
Hypotheses to explain plaque rupture

- Mechanical stress leads to fatigue of the extracellular matrix.
- Oxidised lipoproteins and other reactive oxygen species promote cell death and matrix destruction.
- A change of macrophage phenotype (i.e. stable pattern of gene expression) adversely affects the balance of cap formation and breakdown by excessive matrix degradation and apoptosis of smooth muscle cells.
- A change in smooth muscle phenotype blunts the response to injury.
- Loss of endothelial function leads to increased leukocyte infiltration and reduced smooth muscle cell chemoattractants.
- Entry of other immune cells (T-lymphocytes, mast cells) adversely affects the balance of cap formation and breakdown.

3.1.4. Production of inhibitory mediators

Prostaglandins that elevate cAMP concentrations [199,200], nitric oxide, that elevates cGMP concentration [201–203], interferon-γ [204,205] and TGF-β [86] all antagonise the effects of smooth muscle cell mitogens. For example, vascular endothelial growth factor (VEGF) gene transfer causes NO-mediated reduction of neointima formation after cuff-induced injury in carotid arteries [206]. The pathways are probably diverse; downregulation of early events, such as MAP kinase activity [207] and later events, including cyclin D1 [208] and c-myc [209,210] expression, both contribute. Leucocytes are probably the most important source of all of these inhibitory agents. As shown by Hansson and colleagues, infusion of interferon-γ reduces intimal lesions caused by balloon injury (criterion 1) [206] and these are promoted in lymphocyte-deficient rats (criterion 3) [211]. Similar considerations might apply in atherogenesis. If so, the presence of leucocytes at the shoulder regions of advanced plaque might favour inhibition rather than proliferation of smooth muscle cells. One of the most important antigens for plaque lymphocytes is modified LDL and immunisation of LDL receptor-deficient rabbits with homologous malondialdehyde (MDA)-modified LDL or hypercholesterolaemic rabbits with homologous and oxidized LDL reduces plaque progression [212,213]. This apparently beneficial effect might be a disadvantage at the shoulders of an advanced plaque where fibrous cap formation is critical for stability.

3.1.5. Smooth muscle cell death

Work by Bennett et al. [80,214] has identified that increased susceptibility to apoptosis is an intrinsic characteristic of plaque smooth muscle cells. Although the levels of smooth muscle apoptosis actually present in plaques are controversial [215–219], it probably does occur. Given the slow turnover of smooth muscle cells and the relative rapidity of apoptosis (2–4 h) compared to division (24–48 h), one would predict that the true instantaneous rates of apoptosis are less than those of proliferation (i.e. less that 0.1%), which may be below the noise level of existing methods of detection. These issues are fully discussed elsewhere in this focused issue.

3.1.6. Impaired matrix formation

A further consequence of inadequate smooth muscle cell numbers will be inadequate matrix deposition. Matrix deposition may also be a direct target for the inhibitory action of mediators such as prostaglandins [220] and nitric oxide [221]. Once the matrix becomes devoid of smooth muscle cells, only degradation rather than turnover is possible. Hence, even levels of proteolysis no greater than those present in the evolving plaque might lead to net destruction of the intracellular connective tissue

3.2. New treatments?

Based on our current limited knowledge, caution is needed in proposing new treatments. The debate will undoubtedly be between 'blunderbuss' and 'magic bullet'. Lipid lowering is arguably a blunderbuss approach, affecting as it does most aspects of plaque biology. However, its action at the root of atherogenesis is probably the key to its success. The search for critical molecular mediators of plaque instability will rightly be the holy grail for researchers in this field in the coming years. This quest will be hampered by the lack of appropriate animal models of plaque rupture, although transgenic animals may again provide an answer. Clinical trials will need to be large, protracted and expensive. Nevertheless, recent trials have established that both primary and secondary prevention of myocardial infarction is possible. Greater understanding of the basic biology of fibrous cap formation and rupture promises further significant advances.

Acknowledgements

We thank Dr Mark Bond and Mr Jason Johnson for some of the illustrations. The authors' work is supported by the British Heart Foundation and the Medical Research Council of Great Britain.

References

[1] Davies M, Richardson P, Woolf N, Katz DR, Mann J. Risk of thrombosis in human atherosclerotic plaques: Role of extracellular lipid, macrophage and smooth muscle cell content. Br Heart J 1993;69:377–381.
[2] Falk E, Shah P, Fuster V. Pathogenesis of plaque rupture. In: Fuster V, Ross R, Topol E, editors. Atherosclerosis and coronary artery disease. Philadelphia, New York: Lippincott–Raven, 1996:491–500.
[3] Shah P. Pathophysiology of plaque rupture and the concept of plaque stabilization. Cardiol Clin 1996;14:17–29.
[4] Lee R, Libby P. The unstable atheroma. Arterioscler Thromb Vasc Biol 1997;17:1859–1867.
[5] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801–809.
[6] Ross R, Glomset JA. Atherosclerosis and the arterial smooth muscle cell. Proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. Science 1973;180:1332–1339.
[7] Schwartz S, Campbell G, Campbell J. Replication of smooth muscle cells in vascular disease. Circ Res 1986;58:427–444.
[8] Schwartz S, deBlois D, O'Brien E. The intima. Soil for atherosclerosis and restenosis. Circ Res 1995;77:445–465.
[9] Abedi H, Zachary J. Signalling mechanisms in the regulation of vascular cell migration. Cardiovasc Res 1995;30:544–556.
[10] Weissberg PL, Clesham GJ, Bennett MR. Is vascular smooth-muscle cell-proliferation beneficial? Lancet 1996;347:305–307.
[11] Newby AC, George SJ. Proliferation, migration, matrix turnover, and death of smooth-muscle cells in native coronary and vein graft atherosclerosis. Curr Opin Cardiol 1996;11:574–582.
[12] Orekhov AN, Karpova II, Tertov VV, et al. Cellular composition of atherosclerotic and uninvolved human aortic subendothelial intima: Light microscopic study of dissociated aortic cells. Am J Pathol 1984;115:17–24.
[13] Stary HC. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur Heart J 1990;11(Supple E):3–19.

[14] Kragel AH, Reddy SG, Wittes JT, Roberts WC. Morphometric analysis of the composition of atherosclerotic plaques in the four major epicardial coronary arteries in acute myocardial infarction and in sudden coronary death. Circulation 1989;80:1747–1756.
[15] Stary H, Chandler A, Dinsmore R, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Atherosclerosis, American Heart Association. Arterioscler Thromb Vasc Biol 1995;15:1512–1531.
[16] Anitschkow N, Chalatow S. Uber experimentelle cholesterinsteatose und ihre bendeutung fur die enstehung einiger. Pathologischer prozess. Allg Pathol Anat 1913;24:1–13.
[17] Daley S, Klemp K, Guyton J, Rogers K. Cholesterol-fed and casein-fed rabbit models of atherosclerosis. Part 2: Differing morphological severity of atherogenesis despite matched plasma cholesterol levels. Arterioscler Thromb 1994;14:105–114.
[18] Faggiotto A, Ross R, Harker L. Studies of hypercholesterolaemia in the non-human primate. 1. Changes that lead to fatty streak formation. Arteriosclerosis 1984;4:323–340.
[19] Faggiotto A, Ross R. Studies of hypercholesterolaemia in the non-human primate. 2. Fatty streak conversion to fibrous plaque. Arteriosclerosis 1984;4:341–356.
[20] Masuda J, Ross R. Atherogenesis during low level hypercholesterolemia in the nonhuman primate. 1. Fatty streak formation . Arteriosclerosis 1990;10:164–177.
[21] Masuda J, Ross R. Atherogenesis during low level hypercholesterolemia in the nonhuman primate. 2. Fatty streak conversion to fibrous plaque. Arteriosclerosis 1990;10:178–187.
[22] Watanabe Y. Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL) rabbits. Atherosclerosis 1980;36:261–268.
[23] Buja L, Kita T, Goldstein J, Watanabe Y, Brown M. Cellular pathology of progressive atherosclerosis in the WHHL rabbit — an animal model of familial hypercholesterolemia. Arteriosclerosis 1983;3:87–101.
[24] Nakashima Y, Plump A, Raines E, Breslow J, Ross R. Apo-E-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler Thromb 1994;14:133–140.
[25] Thomas W, Kim D. Atherosclerosis as a hyperplastic and/or neoplastic process. Lab Invest 1983;48:245–255.
[26] Orekhov AN, Andreeva ER, Krushinski AV, et al. Intimal cells and atherosclerosis: Relationship between the number of intimal cells and major manifestations of atherosclerosis in the human heart. Am J Pathol 1986;125:402–415.
[27] Velican C, Velican D. The precursors of coronary atherosclerotic plaques in subjects up to 40 years old. Atherosclerosis 1980;37:33–46.
[28] Aqel NM, Ball RY, Waldmann H, Mitchinson MJ. Identification of macrophages and smooth muscle cells in human atherosclerosis using monoclonal antibodies. J Pathol 1985;146:197–204.
[29] Klurfeld DM. Identification of foam cells in human atherosclerotic lesions as macrophages using monoclonal antibodies. Arch Pathol Lab Med 1985;109:445–449.
[30] Gown AM, Tsukada T, Ross R. Human atherosclerosis: Immunocytochemical analysis of the cellular composition of human atherosclerotic lesions. Am J Pathol 1986;125:191–207.
[31] Poston R, Hussain I. The immunohistochemical heterogeneity of atheroma macrophages:comparison with lymphoid tissues suggest that recently blood-derived macrophages can be distinguished from longer-resident cells. J Histochem Cytochem 1993;41:1503–1512.
[32] Libby P, Geng Y, Aikawa M, et al. Macrophages and atherosclerotic plaque stability. Curr Opin Lipidol 1996;7:330–335.
[33] Nelken N, Coughlin S, Gordon D, Wilcox J. Monocyte chemoattractant protein-1 in human atheromatous plaques. J Clin Invest 1991;88:1121–1127.
[34] Berliner JA, Territo MC, Sevanian A, et al. Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. J Clin Invest 1990;85:1260–1266.
[35] Gordon D, Reidy M, Benditt E, Schwartz S. Cell proliferation in human coronary arteries. Proc Natl Acad Sci USA 1990;87:4600–4604.
[36] Davies MJ, Woolf N. Atherosclerosis in ischaemic heart disease. The mechanisms. London: Science Press, 1990.
[37] Ross R, Masuda J, Raines EW, et al. Localization of PDGF-B protein in macrophages in all phases of atherogenesis. Science 1990;248:1009–1012.
[38] Shimokado K, Raines E, Madtes D, et al. A significant part of macrophage-derived growth factor consists of at least two forms of PDGF. Cell 1985;43:277–286.
[39] Higashiyama S, Abraham J, Klagsbrun M. Heparin-binding EGF-like growth factor stimulation of smooth muscle cell migration: dependence on interactions with cell surface heparan sulphate. J Cell Biol 1993;122:933–940.
[40] Yap AS, Brieher WM, Gumbiner BM. Molecular and functional analysis of cadherin-based adherens junctions. Annu Rev Cell Dev Biol 1997;13:119–146.
[41] Navarro P, Ruco L, Dejana E. Differential localization of VE- and N-cadherins in human endothelial cells: VE-cadherin competes with N-cadherin for junctional localization. J Cell Biol 1998;140:1475–1484.
[42] Gilbertson-Beadling SK, Fisher C. A potential role for N-cadherin in mediating endothelial cell–smooth muscle cell interactions in the rat vasculature. Lab Invest 1993;69:203–209.
[43] Pober JS. Cytokine-mediated activation of vascular endothelium. Am J Pathol 1988;133:426–433.
[44] Kavanaugh WM, Harsh GR, Starksen NF, Rocco CM, Williams LT. Transcriptional regulation of the A and B chain genes of platelet-derived growth factor in microvascular endothelial cells. J Biol Chem 1988;263:1304–1309.
[45] Koo EWY, Gotlieb AI. Endothelial stimulation of intimal cell proliferation in a porcine aortic organ culture. Am J Pathol 1989;134:497–503.
[46] Angelini GD, Soyombo AA, Newby AC. Smooth muscle cell proliferation in response to injury in an organ culture of human saphenous vein. Eur J Vasc Surg 1991;5:5–12.
[47] George SJ, Williams AW, Newby AC. An essential role for platelet-derived growth factor in neointima formation in human saphenous vein in vitro. Atherosclerosis 1996;120:227–240.
[48] Davies M, Woolf N, Rowles P. Morphology of the endothelium over atherosclerotic plaques in human coronary arteries. Br Heart J 1988;60:459–464.
[49] Thompson WD, McGuigan CJ, Snyder C, Keen GA, Smith EB. Mitogenic activity in human atherosclerotic lesions. Atherosclerosis 1987;66:85–93.
[50] Stirk CM, Kochhar A, Smith EB, Thompson WD. Presence of growth stimulating fibrin degradation products containing fragment-E in human atherosclerotic plaques. Atherosclerosis 1993;103:159–169.
[51] Glagov S, Zarins C, Giddens DP, Ku DN. Hemodynamics and atherosclerosis. Arch Pathol Lab Med 1988;112:1018–1031.
[52] Angelini GD, Bryan AJ, Williams HMJ, et al. Timecourse of medial and intimal thickening in pig arteriovenous bypass grafts: relationship to endothelial injury and cholesterol accumulation. J Thorac Cardiovasc Surg 1992;103:1093–1103.
[53] Carmeliet P, Moons L, Lijnen R, et al. Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. Nat Genet 1997;17:439–444.
[54] Shiomi M, Ito T, Tsukada T, Yata T, Ueda M. Cell compositions of coronary and aortic atherosclerotic lesions in WHHL rabbits differ. An immunohistochemical study. Arterioscler Thromb 1994;14:931–937.
[55] Braverman AC. Penetrating atherosclerotic ulcers of the aorta. Curr Opin Cardiol 1994;9:591–597.
[56] Bulkley BH, Hutchins GM. Accelerated atherosclerosis — a morphologic study of 97 saphenous vein coronary artery bypass grafts. Circulation 1977;55:163–169.
[57] Kaartinen M, Penttila A, Kovanen P. Mast cells of two types

differing in neutral protease composition in the human aortic intima. Demonstration of tryptase- and tryptase/chymase-containing mast cells in normal intimas, fatty streaks, and the shoulder region of atheromas. Arterioscler Thromb 1994;14:966–972.
[58] Kaartinen M, Penttila A, Kovanen P. Mast cells in rupture-prone areas of human coronary atheromas produce and store TNF-α. Circulation 1996;94:2787–2792.
[59] Walsh L, Trichieri G, Waldorf H, Whitaker D, Murphy G. Human dermal mast cells contain and release tumor necrosis factor α which induces endothelial leukocyte adhesion molecule 1. Proc Natl Acad Sci USA 1991;88:4220–4224.
[60] Wong G, Goeddel D. Tumor necrosis factor. In: Zembala M, Asherson G, editors. Human monocytes. San Diego, CA: Academic Press, 1989:195–215.
[61] Chamley-Campbell J, Campbell G. What controls smooth muscle phenotype? Atherosclerosis 1981;40:347–357.
[62] Gabbiani G, Kocher O, Bloom W, Vanderkerchove J, Weber K. Actin expression in smooth muscle cells of rat aortic intimal thickening, human atheromatous plaque and cultured rat media. J Clin Invest 1984;73:148–152.
[63] Clowes A, Schwartz S. Significance of quiescent smooth muscle migration in the injured rat carotid artery. Circ Res 1985;56:139–145.
[64] Schwartz S, Reidy M, Clowes A. Kinetics of atherosclerosis. A stem cell model. Ann NY Acad Sci 1985;454:292–304.
[65] Clowes AW, Reidy MA, Clowes MM. Kinetics of cellular proliferation after arterial injury. Smooth muscle growth in the absence of endothelium. Lab Invest 1983;49:327–333.
[66] Holifield B, Helgason T, Jemelka S, et al. Differentiated vascular myocytes: are they involved in neointimal formation? J Clin Invest 1996;97:814–825.
[67] Seidel C. Cellular heterogeneity of the vascular tunica media. Implications for vessel wall repair. Arterioscler Thromb Vasc Biol 1997;17:1868–1871.
[68] Frid MG, Dempsey EC, Durmowitcz AG, Stenmark KR. Smooth muscle cell heterogeneity in pulmonary and systemic vessels: Importance in vascular disease. Arterioscler Thromb Vasc Biol 1997;17:1203–1209.
[69] Kirschenlohr HL, Metcalfe JC, Weissberg PL, Grainger DJ. Proliferation of human aortic vascular smooth muscle cells in culture is modulated by active TGF-beta. Cardiovasc Res 1995;29:848–855.
[70] Kocher O, Gabbiani G. Cytoskeletal features of normal and atheromatous human arterial smooth muscle cells. Hum Pathol 1984;17:875–880.
[71] Wilcox JN, Smith KM, Williams LT, Schwartz SM, Gordon D. Platelet-derived growth factor mRNA detection in human atherosclerotic plaques by in situ hybridization. J Clin Invest 1988;82:1134–1143.
[72] Glukhova M, Kabakov A, Frid M. Modulation of human aorta smooth muscle cell phenotype: a study of muscle-specific variants of vinculin, caldesmon and actin expression. Proc Nat Acad Sci USA 1988;85:9542–9546.
[73] Shi Y, O'Brien J, et al. Adventitial myofibroblasts contribute to neointimal formation in injured porcine coronary arteries. Circulation 1996;94:1655–1664.
[74] Wilcox J, Scott N. Potential role of the adventitia in arteritis and atherosclerosis. Int J Cardiol 1996;54:S21–S35.
[75] Shi Y, O'Brien JE, Mannion JD, et al. Remodeling of autologous saphenous vein grafts — The role of perivascular myofibroblasts. Circulation 1997;95:2684–2693.
[76] Benditt EP, Benditt JM. Evidence for a monoclonal origin of human atherosclerotic plaques. Proc Natl Acad Sci USA 1973;70:1753–1756.
[77] Murry C, Gipaya C, Bartosek T, Benditt E, Schwartz S. Monoclonality of smooth muscle cells in human atherosclerosis. Am J Pathol 1997;151:697–705.
[78] Penn A, Garte SJ, Warren L, Nesta D, Mindich B. Transforming gene in human atherosclerotic plaque DNA. Proc Natl Acad Sci USA 1986;83:7951–7955.
[79] Parkes JL, Cardell RR, Hubbard FC, et al. Cultured human atherosclerotic plaque smooth muscle cells retain transforming potential and display enhanced expression of the *myc* protooncogene. Am J Pathol 1991;138:765–775.
[80] Bennett MR, Evan GI, Schwartz SM. Apoptosis of human vascular smooth muscle cells derived from normal vessels and coronary atherosclerotic plaques. J Clin Invest 1995;95:2266–2274.
[81] Moss N, Benditt E. Human atherosclerotic plaque cells and leiomyoma cells. Am J Pathol 1975;78:175–190.
[82] Newby AC, George SJ. Proposed roles for growth factors in mediating smooth muscle proliferation in vascular pathologies. Cardiovasc Res 1993;27:1173–1183.
[83] Newby A. Molecular and cell biology of native coronary and vein-graft atherosclerosis: regulation of plaque stability and vessel-wall remodelling by growth factors and cell–extracellular matrix interactions. Coron Artery Dis 1997;8:213–224.
[84] Molloy C, Pawlowski J, Taylor D, et al. Thrombin receptor activation elicits rapid tyrosine phosphorylation and stimulation of the Raf-1/MAP kinase pathway preceding delayed mitogenesis in cultured rat aortic smooth muscle cells. Evidence for an obligate autocrine mechanism promoting cell proliferation induced by G protein-coupled receptor agonists. J Clin Invest 1996;97:1173–1183.
[85] Malarkey K, McLees A, Paul A, Gould G, Plevin R. The role of protein kinase C in activation and termination of mitogen-activated protein kinase activity in angiotensin II-stimulated rat aortic smooth-muscle cells. Cell Signal 1996;8:123–129.
[86] Polyak K. Negative regulation of cell growth by TGFβ. Biochim Biophys Acta 1996;1242:185–199.
[87] Heim MH. The Jak-Stat pathway-specific signal transduction from the cell membrane to the nucleus. Eur J Clin Invest 1996;26:1–12.
[88] McCaffrey T, Consigli S, Du B, et al. Decreased type II/type I TGF-β receptor ratio in cells derived from human atherosclerotic lesions. Conversion from an antiproliferative to profibrotic response to TGF-β. J Clin Invest 1995;96:2667–2675.
[89] Björkerud S, Björkerud B. Lipoproteins are major primary mitogens and growth promoters for human arterial smooth muscle cells and lung fibroblasts in vitro. Arterioscler Thromb 1994;14:288–298.
[90] Libby P, Miao P, Ordovas JM, Schaefer EJ. Lipoproteins increase growth of mitogen stimulated arterial smooth muscle cells. J Cell Physiol 1985;124:1–8.
[91] Björkerud B, Björkerud S. Contrary effects of lightly and strongly oxidised LDL with potent promotion of growth versus apoptosis on arterial smooth muscle cells, macrophages and fibroblasts. Arterioscler Thromb Vasc Biol 1996;16:416–424.
[92] Barrett TB, Benditt EP. Platelet-derived growth-factor gene-expression in human atherosclerotic plaques and normal artery wall. Proc Natl Acad Sci USA 1988;85:2810–2814.
[93] Libby P, Warner SJC, Salomon RN, Birinyi LK. Production of platelet-derived growth factor-like mitogen by smooth muscle cells from human atheroma. N Engl J Med 1988;318:1493–1498.
[94] Murry C, Bartosek T, Giachelli C, Alpers C, Schwartz S. Platelet-derived growth factor-A mRNA expression in fetal, normal adult, and atherosclerotic human aortas: Analysis by competitive polymerase chain reaction. Circulation 1996;93:1095–1106.
[95] Rubin K, Tingström A, Hansson GK, et al. Induction of B-type receptors for platelet-derived growth factor in vascular inflammation: possible implications for development of vascular proliferative lesions. Lancet 1988;1:1353–1356.
[96] Wilson V, Ward J, Burnand K, Thomas C. Up-regulation of IGF-1 and collagen-1 messenger-RNA in human atherosclerotic tissue is not accompanied by changes in type-1 IGF receptor or collagen messenger-RNA — an in-situ hybridization study. Coron Artery Dis 1996;7:569–572.
[97] Nikol S, Isner JM, Pickering JG, et al. Expression of transforming growth factor-β1 is increased in human vascular restenosis lesions. J Clin Invest 1992;90:1582–1592.

[98] Liau G, Winkles JA, Cannon MS, Kuo L, Chilian WM. Dietary-induced atherosclerotic lesions have increased levels of acidic FGF messenger-RNA and altered cytoskeletal and extracellular-matrix messenger-RNA expression. J Vasc Res 1993;30:327–332.
[99] Hughes SE. Localization and differential expression of the fibroblast growth-factor receptor (FGFR) multigene family in normal and atherosclerotic human arteries. Cardiovasc Res 1996;32:557–569.
[100] McNamara C, Sarembock I, Gimple L, et al. Thrombin stimulates proliferation of cultured rat aortic smooth muscle cells by a proteolytically activated receptor. J Clin Invest 1993;91:94–98.
[101] Pasche B, Swedenborg J, Hedin U, Olsson P, Ljungqvist A. Thrombin-inhibitory capacity of the injured vessel wall. Thromb Res 1991;62:531–544.
[102] Nelken N, Soifer S, O'Keefe J, et al. Thrombin receptor expression in normal and atherosclerotic human arteries. J Clin Invest 1992;90:1614–1621.
[103] Kowala M, Rose P, Stein P, et al. Selective blockage of the endothelin subtype-A receptor decreases early atherosclerosis in hamsters fed cholesterol. Am J Pathol 1995;146:819–826.
[104] Ferns GAA, Raines EW, Sprugel KH, et al. Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. Science 1991;253:1129–1132.
[105] Lindner V, Reidy MA. Proliferation of smooth muscle cells after vascular injury is inhibited by an antibody against basic fibroblast growth factor. Proc Natl Acad Sci USA 1991;88:3739–3743.
[106] Rutherford C, Martin W, Salame M, et al. Substantial inhibition of neo-intimal response to balloon injury in the rat carotid artery using a combination of antibodies to platelet-derived growth factor-BB and basic fibroblast growth factor. Atherosclerosis 1997;130:45–51.
[107] Wolf Y, Rasmussen L, Ruoslahti E. Antibodies against transforming growth factor-1 suppress intimal hyperplasia in a rat model. J Clin Invest 1994;93:1172–1178.
[108] Fennessy PA, Campbell JH, Campbell GR. An angiotensin-converting enzyme-inhibitor, perindopril, prevents progression of preformed atherosclerotic lesions in the cholesterol-fed rabbit. Clin Sci 1994;87:685–691.
[109] Prescott M, Webb R, Reidy M. Angiotensin-converting enzyme inhibitor versus angiotensin II, AT_1 receptor antagonist: effects on smooth muscle cell migration and proliferation after balloon catheter injury. Am J Pathol 1991;139:1291–1296.
[110] Israel DH, Adams PC, Stein B, Chesebro JH, Fuster V. Antithrombotic therapy in the coronary vein graft patient. Clin Cardiol 1991;14:283–295.
[111] Fingerle J, Kraft T. The induction of smooth muscle cell proliferation in vitro using an organ culture system. Int Angiol 1987;6:65–72.
[112] Soyombo AA, Angelini GD, Bryan AJ, Newby AC. Surgical preparation induces injury and promotes smooth muscle cell proliferation in a culture of human saphenous vein. Cardiovasc Res 1993;27:1961–1967.
[113] Fingerle J, Au YPT, Clowes AW, Reidy MA. Intimal lesion formation in rat carotid arteries after endothelial denudation in absence of medial injury. Arteriosclerosis 1990;10:1082–1087.
[114] Rifkin DB, Moscatelli D. Recent developments in the cell biology of basic fibroblast growth factor. J Cell Biol 1989;109:1–6.
[115] Southgate KM, Davies M, Booth RFG, Newby AC. Involvement of extracellular matrix degrading metalloproteinases in rabbit aortic smooth muscle cell proliferation. Biochem J 1992;288:93–99.
[116] Clowes AW, Clowes MM. Kinetics of cellular proliferation after arterial injury. IV. Heparin inhibits rat smooth muscle mitogenesis and migration. Circ Res 1986;58:839–845.
[117] Campbell JH, Campbell GR. Endothelial cell influences on vascular smooth muscle phenotype. Annu Rev Physiol 1986;48:295–306.
[118] Au YPT, Dobrowolska G, Morris DR, Clowes AW. Heparin decreases activator protein-1 binding to DNA in part by post-translational modification of Jun B. Circ Res 1994;75:15–22.
[119] Wight T, Hascall V. Proteoglycans in primate arteries: III. Characterisation of the proteoglycans synthesized by arterial smooth muscle cells in culture. J Cell Biol 1983;96:167–176.
[120] Kenagy RD, Nikarri ST, Welgus HG, Clowes AW. Heparin inhibits the induction of three matrix metalloproteinases (stromelysin, 92-kDa gelatinase, and collagenase) in primate arterial smooth muscle cells. J Clin Invest 1994;93:1987–1993.
[121] Clowes A, Clowes MM, Kirkman TR, et al. Heparin inhibits the expression of tissue-type plasminogen activator by smooth muscle cells in injured rat carotid artery. Circ Res 1992;70:1128–1136.
[122] Hedin U, Bottger BA, Forsberg E, Johansson S, Thyberg J. Diverse effects of fibronectin and laminin on phenotypic properties of cultured smooth muscle cells. J Cell Biol 1988;107:307–319.
[123] Hedin U, Bottger BA, Lutham J, Johansson S, Thyberg J. A substrate of the cell attachment sequence of fibronectin (Arg–Gly–Asp–Ser) is sufficient to promote transition of arterial smooth muscle cells from a contractile to a synthetic phenotype. Dev Biol 1989;133:489–501.
[124] Thyberg J, Hedin U, Sjölund M, Palmberg L, Bottger BA. Regulation of differentiated properties and proliferation of arterial smooth muscle cells. Arteriosclerosis 1990;10:966–990.
[125] Newby AC, Fabunmi RP, George SJ, et al. Neointimal fibrosis in vascular pathologies: role of growth factors and metalloproteinases in vascular smooth muscle proliferation. Exp Nephrol 1995;3:108–113.
[126] Koyama H, Raines EW, Bornfeldt KE, Roberts JM, Ross R. Fibrillar collagen inhibits arterial smooth muscle proliferation through regulation of CDK2 inhibitors. Cell 1996;87:1069–1078.
[127] Galis ZS, Muszynski M, Sukhova GK, et al. Cytokine-stimulated human vascular smooth muscle cells synthesize a complement of extracellular enzymes required for extracellular matrix degradation. Circ Res 1994;75:181–189.
[128] Sato H, Takino T, Okada Y, et al. A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 1994;370:61–65.
[129] Galis ZS, Kranshöfer R, Fenton JW, Libby P. Thrombin promotes activation of matrix metalloproteinase-2 produced by cultured vascular cells. Arterioscler Thromb Vasc Biol 1997;17:483–489.
[130] Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. J Clin Invest 1996;98:2572–2579.
[131] Fabunmi RP, Baker AH, Murray EJ, Booth RFG, Newby AC. Divergent regulation by growth factors and cytokines of 95-kDa and 72-kDa gelatinases and tissue inhibitors of metalloproteinases-1, -2 and -3 in rabbit aortic smooth muscle cells. Biochem J 1996;315:335–342.
[132] Bendeck MP, Zempo N, Clowes AW, Gelardy RE, Reidy MA. Smooth muscle cell migration and matrix metalloproteinase expression after arterial injury in the rat. Circ Res 1994;75:539–545.
[133] Zempo N, Kenagy RD, Au T, et al. Matrix metalloproteinases of vascular wall cells are increased in balloon-injured rat carotid artery. J Vasc Surg 1994;20:209–217.
[134] Southgate KM, Banning AP, Thurston VJ, et al. Upregulation of basement-membrane-degrading metalloproteinase secretion following balloon angioplasty of pig carotid arteries. Circ Res 1996;79:1177–1187.
[135] George SJ, Zaltsman AB, Newby AC. Surgical preparative injury and neointima formation increase MMP-9 expression and MMP-2 activation in human saphenous vein. Cardiovasc Res 1997;33:447–459.
[136] Zempo N, Koyama N, Kenagy R, Lea H, Clowes A. Regulation of vascular smooth muscle cell migration and proliferation in vitro and injured rat arteries by a synthetic matrix metalloproteinase inhibitor. Arterioscler Thromb Vasc Biol 1996;16:28–33.
[137] Bendeck MP, Irvin C, Reidy MA. Inhibition of matrix metalloproteinase activity inhibits smooth muscle cell migration but not

neointimal thickening after arterial injury. Circ Res 1996;78:38–43.
[138] Forough R, Koyama M, Hasenstab D, et al. Overexpression of tissue inhibitor of metalloproteinase-1 inhibits vascular smooth muscle cell functions in vitro and in vivo. Circ Res 1996;79:812–820.
[139] George S, Johnson J, Angelini G, Newby A, Baker A. Adenovirus-mediated gene transfer of the human TIMP-1 gene inhibits smooth muscle cell migration and neointimal formation in human saphenous vein. Hum Gene Ther 1998;9:867–877.
[140] Bartlett MR, Cowden WB, Parish CR. Differential effects of the antiinflammatory compounds heparin, mannose-6-phosphate, and castanospermine on degradation of the vascular basement membrane by leukocytes, endothelial cells and platelets. J Leukocyte Biol 1995;57:207–213.
[141] Bartlett MR, Underwood PA, Parish CR. Comparative-analysis of the ability of leukocytes, endothelial-cells and platelets to degrade the subendothelial basement-membrane — Evidence for cytokine dependence and detection of a novel sulfatase. Immunol Cell Biol 1995;73:113–124.
[142] Chapman HA, Reise RJ, Shi GP. Emerging roles for cysteine proteases in human biology. Annu Rev Physiol 1997;59:63–88.
[143] Millichip MI, Dallas DJ, Wu EX, Dale S, McKie N. The metallodisintegrin ADAM10 (MADM) from bovine kidney has type IV collagenase activity in vitro. Biochem Biophys Res Commun 1998;245:594–598.
[144] Henney AM, Wakely PR, Davies MJ, et al. Localization of stromelysin gene expression in atherosclerotic plaques by in situ hybridization. Proc Natl Acad Sci USA 1991;88:8154–8158.
[145] Galis ZS, Suhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94:2493–2503.
[146] Galis ZS, Sukhova GK, Kranshöfer R, Clark S, Libby P. Macrophage foam cells from experimental atheroma constitutively express matrix-degrading proteases. Proc Natl Acad Sci USA 1995;92:402–406.
[147] Nikkari ST, O'Brien KD, Ferguson M, et al. Interstitial collagenase (MMP-1) expression in human carotid atherosclerosis. Circulation 1995;92:1393–1398.
[148] Li Z, Li L, Zielke R, et al. Increased expression of 72-kD type IV collagenase (MMP-2) in human aortic atherosclerotic lesions. Am J Pathol 1996;148:121–128.
[149] Zaltsman AB, Newby AC. Increased secretion of gelatinases A and B from the aortas of cholesterol fed rabbits: relationship to lesion severity. Atherosclerosis 1997;130:61–70.
[150] Carmeliet P, Moons L, Dewerchin M, et al. Receptor-independent role of urokinase-type plasminogen activator in pericellular plasmin and matrix metalloproteinase proteolysis during vascular wound healing in mice. J Cell Biol 1998;140:233–245.
[151] van Leeuwen R. Extracellular proteolysis and the migrating vascular smooth muscle cell. Fibrinolysis 1996;10:59–74.
[152] Bornfeldt K, Raines E, Nakano T, et al. Insulin-like growth factor-1 and platelet-derived growth factor-BB induce directed migration of human arterial smooth muscle cells via signalling pathways that are distinct from those of proliferation. J Clin Invest 1994;93:1266–1274.
[153] Ross R. Cellular interactions, growth factors, and atherogenesis. Prev Noninvasive Ther Atheroscler 1990;21:53–58.
[154] Koyama N, Morisaki N, Saito Y, Yoshida S. Regulatory effects of PDGF-AA homodimer on migration of vascular smooth muscle cells. J Biol Chem 1992;267:22806–22812.
[155] Koyama N, Hart CE, Clowes AW. Different effects of platelet-derived growth factor-a and b receptors for the migration and proliferation of cultured baboon smooth muscle cells. Circ Res 1994;75:682–691.
[156] Pauly RR, Bilato C, Sollott SJ, et al. Role of calcium calmodulin dependent protein kinase-II in the regulation of vascular smooth muscle cell migration. Circulation 1995;91:1107–1115.
[157] Bilato C, Pauly RR, Melillo G, et al. Intracellular signaling pathways required for rat vascular smooth muscle cell migration — Interactions between basic fibroblast growth factor and platelet-derived growth factor. J Clin Invest 1995;96:1905–1915.
[158] Olson NE, Chao S, Lindner V, Reidy MA. Intimal smooth muscle cell proliferation after balloon catheter injury: The role of basic fibroblast growth factor. Am J Pathol 1992;140:1017–1023.
[159] Yabkowitz R, Mansfield P, Ryan U, Suchard S. Trombospondin mediates migration and potentiates platelet-derived growth factor-dependent migration of calf pulmonary artery smooth muscle cells. J Cell Physiol 1993;157:24–32.
[160] Kishi J-I, Hayakawa T. Synthesis of latent collagenase and collagenase inhibitor by bovine aortic medial explants and cultured medial smooth muscle cells. Connect Tissue Res 1989;19:63–76.
[161] Casscells W. Migration of smooth muscle and endothelial cells. Critical events in restenosis. Circulation 1992;86:723–729.
[162] Reidy M, Irvin C, Lindner V. Migration of arterial wall cells. Expression of plasminogen activators and inhibitors in injured rat arteries. Circ Res 1996;78:405–414.
[163] Pauly RR, Passaniti A, Bilato C, et al. Migration of cultured vascular smooth muscle cells through a basement membrane barrier requires type IV collagenase activity and is inhibited by cellular differentiation. Circ Res 1994;75:41–45.
[164] Baker A, Zaltsman A, George S, Newby A. Divergent effects of tissue inhibitor of metalloproteinase-1, -2, or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death in vitro. J Clin Invest 1998;101:1478–1487.
[165] Kenagy R, Hart C, Stetler-Stevenson W, Clowes A. Primate smooth muscle cell migration from aortic explants is mediated by endogenous platelet-derived growth factor and basic fibroblast growth factor acting through matrix metalloproteinases 2 and 9. Circulation 1997;96:3555–3560.
[166] Clowes AW, Clowes MM, Reidy MA, Belin D. Smooth muscle cells express urokinase during mitogenesis and tissue-type plasminogen activator during migration in injured rat carotid artery. Circ Res 1990;67:61–67.
[167] Jackson CL, Reidy MA. The role of plasminogen activation in smooth muscle cell migration after arterial injury. Ann NY Acad Sci 1992;667:141–150.
[168] Jackson CL, Raines EW, Ross R, Reidy MA. Role of endogenous platelet-derived growth factor in arterial smooth muscle cell migration after balloon catheter injury. Arterioscler Thromb 1993;1993:13.
[169] Carmeliet P, Moons L, Lijnen R, et al. Inhibitory role of plasminogen activator inhibitor-1 in arterial wound healing and neointima formation. A gene targeting and gene transfer study in mice. Circulation 1997;96:3180–3191.
[170] Ang AH, Tachas G, Campbell JH, Bateman JF, Campbell GR. Collagen synthesis by cultured rabbit aortic smooth muscle cells. Biochem J 1990;265:461–469.
[171] Thyberg J. Differentiated properties and proliferation of arterial smooth muscle cells in culture. Int Rev Cytol 1996;169:183–265.
[172] Savani R, Wang C, Yang B, et al. Migration of bovine aortic smooth muscle cells after wounding injury — the role of hyaluronan and rhamm. J Clin Invest 1995;95:1158–1168.
[173] Wight T. The extracellular matrix and atherosclerosis. Curr Opin Lipidol 1995;6:326–334.
[174] Thyberg J, Blomgren K, Roy J, Tran PK, Hedin U. Phenotypic modulation of smooth muscle cells after arterial injury is associated with changes in the distribution of laminin and fibronectin. J Histochem Cytochem 1997;45:837–846.
[175] Koyama N, Koshikawa T, Morisaki N, Saito Y, Yoshida S. Secretion of a potent new migration factor for smooth muscle cells (SMC) by cultured SMC. Atherosclerosis 1991;86:219–226.
[176] DiMilla P, Stone J, Quinn J, Albelda S, Lauffenburger D. Maximal

migration of human smooth muscle cells on fibronectin and type IV collagen occurs at an intermediate attachment strength. J Cell Biol 1993;122:729–737.
[177] Liaw L, Slinner M, Raines E, et al. The adhesive and migratory effects of osteopontin are mediated via distinct cell surface integrins: role of $\alpha_v\beta_3$ in smooth muscle migration to osteopontin in vitro. J Clin Invest 1995;95:713–724.
[178] Skinner MP, Raines EW, Ross R. Dynamic expression of alpha-1–beta-1 and alpha-2–beta-1 integrin receptors by human vascular smooth muscle cells —- alpha-2–beta-1 integrin is required for chemotaxis across type-I collagen coated membranes. Am J Pathol 1994;145:1070–1081.
[179] Naito M, Hayasi T, Funaki C, et al. Vitronectin-induced haptotaxis of vascular smooth muscle cells in vitro. Exp Cell Res 1991;194:154–156.
[180] Brown E, Landgren C, Nordt T, Fujii S. Stimulation of migration of human aortic smooth muscle cells by vitronectin: implications for atherosclerosis. Cardiovasc Res 1994;28:1815–1820.
[181] Wight T. Cell biology of arterial proteoglycans. Arteriosclerosis 1989;9:1–20.
[182] Shekhonin B, Domogatsky S, Idelson G, Koteliansky V, Rukosuerm VS. Relative distribution of fibronectin and type I, III, IV, V collagens in normal and atherosclerotic intima of human arteries. Atherosclerosis 1987;67:9–16.
[183] Murata K, Motoyama T, Kotake C. Collagen types in various layers of the human aorta and their changes with the atherosclerotic process. Atherosclerosis 1986;60:251–262.
[184] Rekhter M, Zhang K, Narayanan A, et al. Type-1 collagen gene-expression in human atherosclerosis — localization to specific plaque regions. Am J Pathol 1993;143:1634–1648.
[185] Rekhter M, Gordon D. Cell proliferation and collagen synthesis are two independent events in human atherosclerotic plaques. J Vasc Res 1994;31:280–286.
[186] Roberts A, Sporn M, Assoian R, et al. Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci USA 1986;83:4167–4171.
[187] Ignoltz R, Massague J. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 1986;261:4337–4345.
[188] Bendeck M, Regenass S, Tom W, et al. Differential expression of α1 type VIII collagen in injured platelet-derived growth factor-BB-stimulated rat carotid arteries. Circ Res 1996;79:524–531.
[189] Shanahan CM, Weissberg PL, Metcalfe JC. Isolation of gene markers of differentiated and proliferating vascular smooth muscle cells. Circ Res 1993;73:193–204.
[190] Giachelli CM, Bae N, Almeida M, et al. Osteopontin is elevated during neointima formation in rat arteries and is a novel component of human atherosclerotic plaques. J Clin Invest 1993;92:1686–1696.
[191] Majack RA, Cook SC, Bornstein P. Control of smooth muscle cell growth by components of the extracellular matrix: autocrine role for thrombospondin. Proc Natl Acad Sci USA 1986;83:9050–9054.
[192] Matsuno H, Stassen J, Vermylen J, Deckmyn H. Inhibition of integrin function by a cyclic RGD-containing peptide prevents neointima formation. Circulation 1994;90:2203–2206.
[193] Srivatsa S, Fitzpatrick L, Tsao P, et al. Selective αvβ3 integrin blockade potently limits neointimal hyperplasia and lumen stenosis following deep coronary arterial stent injury: evidence for the functional importance of integrin αvβ3 and osteopontin expression during neointima formation. Cardiovasc Res 1997;36:408–428.
[194] Lendon CL, Davies MJ, Born GVR, Richardson PD. Atherosclerotic plaque caps are locally weakened when macrophage density is increased. Atherosclerosis 1991;87:87–91.
[195] Mann JM, Davies MJ. Vulnerable plaque. Relation of characteristics to degree of stenosis in human coronary arteries. Circulation 1996;94:928–931.
[196] Libby P. Molecular basis of the acute coronary syndromes. Circulation 1995;91:2844–2850.
[197] Libby P, Sukhova G, Lee RT, Galis ZS. Cytokines regulate vascular functions related to stability of the atherosclerotic plaque. J Cardiovasc Pharmacol 1995;25:S9–S12.
[198] Lindner V, Majack RA, Reidy MA. Basic fibroblast growth factor stimulates endothelial regrowth and proliferation in denuded arteries. J Clin Invest 1990;85:2004–2008.
[199] Kerr L, Olashaw N, Matrisian L. Transforming growth factor β1 and cAMP inhibit transcription of epidermal growth factor- and oncogene-induced transin RNA. J Biol Chem 1988;263:16999–17005.
[200] Motoyama Y, Seki J, Katoh Y, Nishio M, Yoshida K. Effect of TFC-612, a 7-thia prostaglandin E1 derivative, on intimal thickening after endothelial injury with balloon catheter in rats. Atherosclerosis 1994;108:159–165.
[201] Garg UC, Hassid A. Nitric oxide-generating vasodilators inhibit mitogenesis and proliferation of BALB/C 3T3 fibroblasts by a cyclic GMP-dependent mechanism. Biochem Biophys Res Commun 1990;171:474–479.
[202] Kariya K, Kawahara Y, Araki S-I, Fukuzaki H, Takai Y. Antiproliferative action of cyclic GMP-elevating vasodilators in cultured rabbit aortic smooth muscle cells. Atherosclerosis 1989;80:143–147.
[203] Assender JWA, Southgate KM, Hallett MB, Newby AC. Inhibition of proliferation but not Ca^{2+} mobilization by cAMP and cGMP in rabbit aortic smooth muscle cells. Biochem J 1992;288:527–532.
[204] Hansson GK, Hellstrand M, Rymo L, Rubbia L, Gabbiani G. Interferon-gamma inhibits both proliferation and expression of differentiation specific alpha-smooth muscle actin in arterial smooth muscle cells. J Exp Med 1989;170:1595–1608.
[205] Hansson GK, Holm J. Interferon-γ inhibits arterial stenosis after injury. Circulation 1991;84:1266–1272.
[206] Laitinen M, Zachary I, Breier G, et al. VEGF gene transfer reduces intimal thickening via increased production of nitric oxide in carotid arteries. Hum Gene Ther 1997;8:1737–1744.
[207] Graves LM, Bornfeldt KE, Raines EW, et al. Protein kinase A antagonizes platelet-derived growth factor signaling by mitogen-activated protein kinase in human arterial smooth muscle cells. Proc Natl Acad Sci USA 1993;90:10300–10304.
[208] L'Allemain G, Lavoie J, Rivard N, Baldin V, Pouyssegur J. Cyclin D1 expression is a major target of the cAMP-induced inhibition of cell cycle entry in fibroblasts. Oncogene 1997;14:1981–1990.
[209] Bennett MR, Littlewood TD, Hancock DC, Evan GI, Newby AC. Down-regulation of the *c-myc* proto-oncogene in arrest of rat aortic vascular smooth muscle cell proliferation — different effects of mitogen withdrawal, heparin, interferon-gamma and cyclic nucleotide analogues. Biochem J 1994;302:701–708.
[210] Bennett MR, Evan GI, Newby AC. Deregulated expression of the *c-myc* oncogene abolishes inhibition of proliferation of rat vascular smooth muscle cells by serum reduction, interferon-γ, heparin and cyclic nucleotide analogues and induces apoptosis. Circ Res 1994;74:525–536.
[211] Hansson G, Stemme S, Geng Y, Holm J. Can immunocompetent cells and their cytokines play a role in atherogenesis? Nouv Rev Fr Hematol 1992;34:543–546.
[212] Palinski W, Miller E, Witztum J. Immunization of low-density-lipoprotein (LDL) receptor deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. Proc Natl Acad Sci USA 1995;92:821–825.
[213] Ameli S, Hultgardhnilsson A, Regnstrom J, et al. Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. Arterioscler Thromb Vasc Biol 1996;16:1074–1079.
[214] Bennett M, Macdonald K, Chan S, Boyle J, Weissberg P. Cooperative interaction between RB and p53 regulate cell proliferation, cell senescence, and apoptosis in human vascular smooth

muscle cells from atherosclerosis plaques. Circ Res 1998;82:704–712.
[215] Isner JM, Kearney M, Bortman S, Passeri J. Apoptosis in human atherosclerosis and restenosis. Circulation 1995;91:2703–2711.
[216] Han DKM, Haudenschild CC, Hong MK, et al. Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. Am J Pathol 1995;147:267–277.
[217] Geng YJ, Libby P. Evidence for apoptosis in advanced human atheroma — colocalization with interleukin-1-beta-converting enzyme. Am J Pathol 1995;147:251–266.
[218] Kockx MM, Muhring J, Bortier H, Demeyer GRY, Jacob W. Biotin-conjugated or digoxigenin-conjugated nucleotides bind to matrix vesicles in atherosclerotic plaques. Am J Pathol 1996;148:1771–1777.
[219] Kockx MM, Muhring J, Knaapen MWM, deMeyer GRY. RNA synthesis and splicing interferes with DNA in situ end labeling techniques used to detect apoptosis. Am J Pathol 1998;152:885–888.
[220] Yu H, Gallagher AM, Garfin PM, Printz MP. Prostacyclin release by rat cardiac fibroblasts — Inhibition of collagen expression. Hypertension 1997;30:1047–1053.
[221] Kolpakov V, Gordon D, Kulik T. Nitric oxide-generating compounds inhibit total protein and collagen-synthesis in cultured vascular smooth muscle cells. Circ Res 1995;76:305–309.