Dual Role of Matrix Metalloproteinases (Matrixins) in Intimal Thickening and Atherosclerotic Plaque Rupture

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part by soluble cytokines and cell-cell interactions. Activation of MMP proforms requires other MMPs or other classes of protease. MMP activation contributes to intimal growth and vessel wall remodeling in response to injury, most notably by promoting migration of vascular smooth muscle cells. A broader spectrum and/or higher level of MMP activation, especially associated with inflammation, could contribute to pathological matrix destruction and plaque rupture. Inhibiting the activity of specific MMPs or preventing their upregulation could ameliorate intimal thickening and prevent myocardial infarction.

I. INTRODUCTION

Already in the middle of the 19th century, Virchow (282) described the key histological feature of the thickened intima in atherosclerosis, namely, the presence of cholesterol as extracellular crystals and in “foam cells.” He formulated the “infiltration” hypothesis, which is still current, stating that atherosclerosis results from the infiltration of lipids and inflammatory cells from the blood. The characterization of intimal expansion at the molecular level has continued to the present day and is summarized as background in section II of this review.

Based on Nobel Prize winning work by Goldstein and Brown into the mechanisms of cholesterol uptake into cells, Steinberg et al. (263) formulated the “modified low-density lipoprotein” (LDL) hypothesis. According to this, modifications, including importantly oxidation, increase the uptake of plasma LDL into macrophages and give rise to foam cells. In 1972 Ross and Glomset (229) initially formulated the “response to injury” hypothesis, which focused on the behavior of vascular smooth muscle (VSMC) and endothelial cells (EC) and took into account the recent discovery of peptide growth factors. According to this proposal and subsequent modifications, the vessel wall responded by endothelial dysfunction and VSMC proliferation when injured mechanically, by abnormal flow, or by other noxious agents, such as cigarette smoke or glycated proteins produced in diabetic patients. Response to injury provided a satisfactory explanation for fibrous cap formation, for the localization of atherosclerosis in areas of disturbed flow and many risk factors in addition to hypercholesterolemia. The hypothesis also offered an explanation for the iatrogenic intimal thickening that had begun to be recognized as a clinical problem following the introduction of coronary bypass grafting, coronary angioplasty, and heart transplantation. Another consequence of all these perturbations is inflammation, and Libby (157) championed the possible pathogenetic role of inflammatory mediators. Hansson and Wick (reviewed in Ref. 107) formulated convincing cases for an immune component of atherosclerosis. In his later reviews, Ross (228) largely succeeded in unifying these various hypotheses by proposing oxidized lipids as one important protagonist of chronic vascular inflammation and hence response to injury.

By the beginning of the 1990s, a long list of inflammatory cytokines and growth factors that might mediate the response to injury had been identified (198). When examined in isolated tissue culture, many of these cytokines promoted the proliferation of VSMC, and a smaller subset affected migration. However, when these observations were translated into animal models of neointima formation by Reidy and Clowes’ groups (58) or human organ culture models (our work) (259), it was apparent that growth factors by themselves were insufficient and that extracellular proteases might also be needed (58). Parallels between invasion of VSMC in the neointima and invasion of cancer cells and other a priori reasons itemized in section III of this review led us to consider a role for matrix metalloproteinases (MMPs) (256). Section IV provides a necessary introduction to the MMP family members that have subsequently been discovered in the vessel wall. Section V attempts to structure the wealth of data regarding the physiological and pathological regulation of MMP activity in the vasculature. A comprehensive and critical examination of the evidence for involvement of MMPs in intimal thickening is one of the major tasks of this review. Section VI lists the criteria, and section VII details the evidence.

Thanks mainly to careful autopsy observations, particularly by Davies and Thomas (64, 65) and Falk et al. (77), it has become accepted that myocardial infarction is a distinct acute pathological event superimposed on the chronic process of atherosclerosis. Two main mechanisms were identified, most often rupture of the fibrous cap (“plaque rupture”) or alternatively disruption of the surface endothelium (“plaque erosion”) (reviewed in Ref. 63). Plaque rupture was associated inter alia with a greater degree of inflammation and destruction of the extracellular matrix (152). This led Henney et al. (113) and Libby and co-workers (90) independently to propose a pathogenetic role for MMPs in plaque rupture. A detailed critical appraisal of this concept is presented in section IX. Section X summarizes the dual role of MMPs in intimal expansion and plaque rupture and asks how these two roles, which appear to have opposite effects on plaque stability, can be reconciled.

II. INTIMAL THICKENING: FROM PHYSIOLOGICAL ADAPTATION TO PATHOLOGICAL FAILURE

The normal intima of large arteries has a continuous endothelial monolayer seated on a basement membrane.
In humans, the intima is focally thickened by a hyaluronan-rich matrix with sparse VSMC (261). All basement membranes contain type IV collagen, laminin, and heparan sulfate proteoglycans, such as perlecain (276) and syndecans (298). The normal media contains contractile VSMC surrounded by their own basement membrane (275), a few resident macrophages, and possibly fibroblasts (308). The medial interstitial matrix contains types I and III collagen, a variety of glycoproteins, including fibronectin, vitronectin, tenasin, and thrombospondin, together with chondroitin/dermatan sulfate proteoglycans, such as versican (197). There is also elastin arranged into distinct layers (lamellae). The adventitia contains fibroblasts, small blood vessels, and fat in a loose interstitial matrix (293, 294).

Intimal growth is mediated by an increase in the number and diversity of cells and is accompanied by accumulation of new extracellular matrix (57). It is an adaptive or repair response to a variety of adverse physical and biochemical stimuli acting on the vessel wall. For example, when pulmonary hypertension occurs secondarily to congenital or acquired abnormalities of the heart, VSMC hyperplasia is a direct response to increased tangential wall stress (289). A similar response occurs also in arteriovenous fistulas and surgical vein-grafts into the systemic circulation (10, 70). Mechanical injury, particularly to the luminal surface of arteries during percutaneous transluminal angioplasty, also triggers VSMC hyperplasia as a repair response, at least in healthy animals’ arteries (27, 28). Intimal remodeling is almost always accompanied by transient inflammation. For example, immunologically driven processes, such as transplant atherosclerosis (232) and foreign body reaction to stent implantation after angioplasty (79, 138, 281), provoke intimal thickening.

The most common and arguably most complex chemical insult that provokes intimal expansion is atherosclerosis. Deposition of LDL, which subsequently becomes oxidized, is the most likely initial event. This is followed by infiltration of circulating monocytes, which convert to macrophages and take up oxidized LDL to become foam cells (228). Activated lymphocytes also occur in atherosclerotic plaques at all stages of its progression, and other inflammatory cells including mast cells are also present (107). Formation of a distinct fibrous cap over a large lipid core occurs only as a late consequence of atherosclerosis (261). The lipid core arises because macrophages die by apoptosis and spill their lipid contents. The plaque cap could form by migration of typical, contractile VSMC from the media, which would account for the frequent medial wasting observed at the base of atherosclerotic plaques (60). However, this paradigm has been challenged recently. First, many plaque caps appear to have arisen by clonal expansion of one or a few cells (26, 194). This could arise by selection of a subpopulation of medial VSMC that express a proliferative phenotype (108) or expansion from circulating VSMC precursors (stem cells) that have invaded the plaque (44, 118, 235). Alternatively, it has been suggested that some neointimal VSMC may be transdifferentiated adventitial fibroblasts (308). The atherosclerotic intima also contains capillaries, formation of which contributes to plaque growth in animal models (193).

If intimal growth is such that the initial lumen is narrowed by >70%, distal blood flow is restricted and chronic tissue ischemia results. This occurs in native coronary arteries and during restenosis after coronary angioplasty or failure of some coronary vein grafts: it then results in stable angina pectoris. Constrictive vessel wall remodeling (shrinking of the whole vessel wall), which involves the fibroblast-rich adventitia as well as the media, may exacerbate the unwanted flow restriction caused by intimal thickening (71, 188). Conversely, expansive remodeling, i.e., enlargement of the total vessel wall cross-sectional area, ameliorates these adverse hemodynamic consequences (97). On the other hand, pathological outward expansion, for example, in aortic aneurysms, can lead to catastrophic medial dissection and rupture. In humans, aortic aneurysms are considered the consequence of invasion of atherosclerotic inflammation into the media (45, 224). Recent histological and in vivo intravascular ultrasound studies showed that ruptured plaques are more likely to have undergone outward remodeling (209, 240). Moreover, apolipoproteinE (ApoE) knockout mice show frequent aortic microaneurysms at the base of atherosclerotic plaques (47). It is tempting, therefore, to conclude, as Pasterkamp et al. did (209), that aneurysmal dilatation, expansive remodeling, and plaque rupture actually have a common inflammatory origin and can be regarded as aspects of the same phenomenon.

Catastrophic rupture or surface erosion of the fibrous cap underlies fatal coronary thrombosis and myocardial infarction (MI), which is the leading cause of premature death in developed countries (see http://www.who.int/whosis/). Plaque rupture, which accounts for more than 80% of fatal MI in men, occurs in regions of high tangential stress and where collagen is depleted (63). The implication is that matrix destruction weakens the plaque to the point where it can no longer resist the cyclical strain caused by the cardiac cycle (158). Plaque erosion accounts for as much as 50% of MI in young women (63). It is apparently a distinct pathology triggered by large-scale loss of the endothelium and exposure of the thrombogenic underlying basement membrane. Because much less is known about its underlying basis, it will not be considered further in this review.
III. WHY CONSIDER A ROLE FOR MATRIX METALLOPROTEINASES IN INTIMAL EXPANSION AND PLAQUE RUPTURE?

The following four arguments represent the a priori case for the involvement of MMPs in intimal thickening and plaque rupture.

Intimal thickening constitutes the generation of new tissue at least in part using VSMC derived from the media. As illustrated in Figure 1, MMPs could catalyze removal of the basement membrane around VSMC and facilitate contacts with the interstitial matrix. This could promote a change from quiescent, contractile VSMC to cells capable of migrating and proliferating to mediate repair. Freeing of sequestered growth factors and production of new, permissive matrix components could further promote VSMC migration and proliferation.

Plaque rupture results from net destruction of the intimal extracellular matrix (ECM), which must be driven by proteases.

Remodeling of the vascular ECM occurs at neutral pH, where MMPs are active.

The MMPs (also known as matrixins) are a large family of proteases that each has at least one ECM component as a putative substrate.

IV. MATRIX METALLOPROTEINASE EXPRESSED IN THE MAMMALIAN VASCULATURE

The mammalian MMPs (also known as matrixins) are a family of at least 25 secreted or surface-bound proteases (264, 283), of which 14 have been characterized in vascular cells (Fig. 2). They belong to the subfamily of metallopeptidases known as metzincins because their active site contains an essential Zn$^{2+}$. Their activity can be regulated at four levels: induction of MMP genes, vesicle trafficking and secretion, activation of latent proforms, and complexing with specific tissue inhibitors of metalloproteinases (TIMPs).

Structurally, MMPs comprise a signal sequence, a prodomain, a catalytic domain, and usually a hemopexin-like domain, although this is absent in MMPs-7 and -26 and replaced with an immunoglobulin-like domain in MMP-23 (Fig. 2). Most MMPs are expressed as inactive, latent proforms, although MMP-11, -21, -23, and -27 and the membrane-type MMPs (MT-MMPs) are activated by...
removal of the prodomain by furins in the endosomal pathway. Except in MMP-23, latency is maintained because a conserved cysteine residue in the prodomain sequence PRCGXPD displaces the catalytic water molecule from the active site zinc ion (260). In the test tube MMPs can be activated by physical (e.g., low pH, binding of sodium dodecyl sulfate) or chemical (e.g., 4-aminophenylmercuric acid) modification, which provokes structural unwinding, autocatalytic cleavage of the NH₂-terminal prodomain and hence opens the so-called “cysteine switch” (260). A physiological example of chemical activation may be the ability of nitric oxide to activate MMP-9 in the brain (102). Reactive oxygen species (ROS) potentially released from macrophages can also activate MMP-2 and -9 (220).

Most pro-MMPs are likely to be activated biologically by tissue or plasma proteinases, including other MMPs (Fig. 3). One well-established case is activation of MMP-2

FIG. 2. Structure of MMPs: variations on a theme. The domain structure common to MMPs and variations on this in individual MMPs are shown. Tm represents the transmembrane domain, while hexagonP represents a glycosylphosphatidylinositol (GPI)-anchor membrane anchor.

FIG. 3. Hypothetical activation cascades for MMPs. Up to four levels of catalytic activation of MMPs may be responsible for their ultimate proteolytic effect on matrix components. Lysosomal cathepsins might provide a link between tissue injury and increased proteolysis. Tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) as well as reactive oxygen species (ROS) and nitric oxide (NO) are also implicated.
at the cell surface by MT-MMPs, which is best understood for MT1-MMP (264, 267). TIMP-2 bound to the catalytic site of one MT1-MMP molecule acts as a receptor for MMP-2 by interacting with the noncatalytic COOH-terminal domain. A second molecule of MT1-MMP then partially cleaves the MMP-2 prodomain, which is then fully cleaved by autoproteolysis. None of the other TIMPs can substitute for TIMP-2 during the activation of MMP-2 (299). One consequence is that MT-MMP-mediated MMP-2 activation is functionally impaired in TIMP-2 knockout mice (264). Secreted MMPs, particularly, MMPs-3, -7, and -10, can activate other secreted MMPs (283). Serine proteases, such as trypsin and mast cell-derived chymase and tryptase, activate several MMPs in the test tube (149). Hence, the mast cell enzymes could be significant activators of MMPs in atherosclerotic plaques in vivo (131). The serine proteases, thrombin (87, 145), and Factor Xa (223) promote activation of MMP-2, which suggests a link between thrombosis and activation of ECM turnover. Tissue but not plasma kallikrein activates purified MMP-9 (69). The abundant serine protease, plasmin, activates pro-MMPs-1, -3, -7, -9, -10, and -13 in vitro (159). Moreover, plasminogen knockout in mice impairs MMP-9-mediated elastin degradation and aneurysm formation, showing that plasmin is an essential MMP activator in vivo (47). Plasmin is, in turn, activated by plasminogen activators that can be secreted or located at the cell surface by binding to specific receptors (74). From knockout studies, the urokinase-type plasminogen activator seems to be particularly important for vascular remodeling (46). This must be activated by yet other proteases, perhaps plasma kallikrein (74). Hence, MMP activation is likely to require a complex cascade of catalytic activation during which the final proteolytic effect could be greatly amplified (Fig. 3). The question arises as to which processes initiate the cascade during vascular injury. Possibilities worthy of consideration are release of lysosomal proteases during cell death or production of ROS during oxidative stress (220).

The MMP catalytic domain contains the canonical zinc-binding sequence HEXxGxHxxGxxHS. Uniquely in the gelatinases, MMP-2 and -9, the catalytic domain is interrupted by three repeats of a fibronectin type II-like sequence. The COOH-terminal hemopexin domain in MMP-1 aids in recognition of large matrix molecule substrates and is important in dimerization and in interaction of other pro-MMPs with TIMPs (283). Such remote interactions between MMP-9 and TIMP-1 or MMP-2 and TIMP-2 alter the kinetics of activation but do not inhibit catalytic activity. The hemopexin domain in MMP-2 also functions to anchor the enzyme to the cell membrane via integrin binding (37). Similar mechanisms may underlie integrin binding of MMP-1 and binding of MMP-9 to the CD44 hyaluronan receptor (264). The MT-MMPs are intrinsic membrane proteins; MMPs-14 to -16 and -24 have a COOH-terminal transmembrane domain while MMPs-17 and -25 have a glycosphatidylinositol anchor.

A variety of protein inhibitors of MMP have been described, among which the TIMPs are by far the most potent and selective (15). There are at least four members of the TIMP family that form tight inhibitory 1:1 complexes with MMPs. These interactions are generally rather nonselective, although, exceptionally, TIMP-1 is a much weaker inhibitor of MT-MMPs than other TIMPs (248, 295). TIMPs-1 to -4 are each made up of two domains containing three disulfide bonds (195). The NH2-terminal cysteine is particularly important in inhibition, since its free α-amino group and carbonyl function displace the catalytic water molecule from the essential zinc ion in the MMP active site (99). The three COOH-terminal loops of TIMPs mediate complex formation with the progelatinase hemopexin domain and are largely responsible for the unique matrix binding capacity of TIMP-3 (146). The importance of the COOH terminus of TIMP-3 is further underlined by the fact that any of six mutations that generate a free SH-group in this portion of the molecule leads to the autosomal-dominant blindness, Sorsby’s fund dystrophy (288). In plasma, the general protease inhibitor α2-macroglobulin may also be important. The physiological relevance of other inhibitors including the RECK protein (204) is unknown.

Disintegrin metalloproteinases (ADAMs) also contain an MMP-like catalytic domain, which in some cases remains catalytically active. In general, TIMPs do not bind to or inhibit the catalytic site of ADAMs, although TIMP-3 inhibits a number of ADAMs and TIMP-1 inhibits ADAM-10 (8). Of the more than 30 members of this family, only ADAM-10 (36), ADAM-15 (115), and ADAM-17 (31) have been characterized in vascular cells. ADAMs will not be discussed further, since there is insufficient evidence to decide whether or not they have a specific role in intimal thickening or plaque rupture.

Based on substrate specificity and structural homology, MMPs can be assigned to five subgroups: interstitial collagenases, gelatinases, stromelysins/matrilysins, membrane-type MMPs (MT-MMPs), and others (Table 1). MMPs appear to have distinct but overlapping specificities against purified matrix proteins in the test tube (see Refs. 264, 283). Interstitial collagenases (MMP-1, MMP-8, and MMP-13) cleave intact fibrillar interstitial collagens I, II, and III, and MMP-14, an MT-MMP, also has this ability (205). The gelatinases (MMP-2 and -9) have prominent activity against basement membrane components including type IV collagen and laminin and also elastin (5, 134). Stromelysins-1 and -2 (MMPs-3 and -10) and matrilysins (MMP-7) have a wide substrate repertoire, which includes most of the ECM proteins and proteoglycans except intact fibrillar collagens. Stromelysin-3 (MMP-11) is related in sequence to the other stromelysins, but its substrate specificity is unusual. Its main substrates may be serum pro-
tease inhibitors, not matrix proteins (241, 264). Metalloelastase (MMP-12) is usually set apart from the other groups of MMPs on sequence criteria and because of its limited activity as a collagenase (264). The MT-MMPs were first identified by their ability to activate progelatinase A (236), although MMP-17 does not, and all of the MT-MMPs also cleave at least one matrix protein (283). MMPs also cleave a variety of nonmatrix substrates (for comprehensive listings, see Refs. 264, 283).

From the substrate specificities defined in vitro (264, 283), it appears that individual MMPs have only a limited and inefficient capacity to remodel the ECM. However, acting together, they could efficiently degrade all of the major protein and proteoglycan-core-protein components (195, 283). The ability of some MMPs to activate the proforms of others, as discussed above (Fig. 3), further suggests that individual MMPs need to act together and with other classes of protease to achieve matrix turnover.

V. CRITERIA FOR MATRIX METALLOPROTEINASE INVOLVEMENT IN INTIMAL THICKENING AND PLAQUE RUPTURE

A convincing case should consist of the following elements.

1) MMPs are expressed and regulated in vascular cells.

2) MMP gene expression and activity are temporally and spatially related to intimal expansion and plaque rupture.

3) Introduction of MMP genes mimics the events of matrix remodeling.

4) Inhibition or knockout of MMP genes suppresses remodeling.

5) Plausible mechanisms explain the actions of MMPs.

VI. MATRIX METALLOPROTEINASE AND TISSUE INHIBITOR OF METALLOPROTEINASE GENE TRANSCRIPTION IS DIFFERENTIALLY REGULATED IN ISOLATED VASCULAR CELLS IN CULTURE: MATRIX METALLOPROTEINASE UPREGULATION MAY OCCUR IN SEVERAL DISTINCT STAGES

A. VSMC

Upregulation of MMP production from VSMC fits the pattern of a series of stages (Fig. 4). VSMC constitutively express the gelatinase MMP-2 (88, 211, 256, 304). Stretch further upregulates MMP-2 via production of ROS in an NAD(P)H oxidase-dependent manner (101). Upregulation of MMP-2 production and dramatic induction of the other gelatinase, MMP-9, occur rapidly after mechanical injury (25, 95, 125, 257). These findings imply that stretch and injury can promote MMP-mediated breakdown of basement membranes, for which there is direct evidence (1). MMP-2 and -9 also have significant elastolytic activity that could be important in remodeling of arteries in response to altered hemodynamics (55). Inflammatory cytokines, such as interleukin (IL)-1, IL-4 and tumor necrosis factor-α (TNF-α), coordinate induction of a broad range of MMPs, including MMPs-1, -3, and -9 (88, 147, 234). Cytokines act synergistically with growth factors, such as platelet-derived growth factor (PDGF) and fibroblast growth factor-2 (FGF-2) (34, 75, 137, 213, 304). The presence of both inflammatory mediators and growth factors in injured and atherosclerotic blood vessels could therefore drive the production of MMPs with the ability to efficiently remodel both basement membranes and many components of the interstitial matrix. Cell contact with T-lymphocyte membranes and addition of recombinant CD40 ligand induce MMPs-1, -3, -8, and -9 in VSMC (114, 241, 243), which implies a relationship between immune activation and wide-ranging matrix remodeling.

The regulation of TIMPs shows a distinctly different pattern from MMPs. TIMP-1 is either constitutive (75, 88) or upregulated by the fibrogenic cytokines, PDGF, and transforming growth factor-β (TGF-β) (76). TIMP-2 secretion is constitutive (75, 88), while TIMP-3 expression is upregulated by a combination PDGF and TGF-β (75).

Together these findings suggest that stretch, injury, inflammation, and immune activation progressively move the MMP/TIMP balance in VSMC towards proteolysis, while fibrogenic stimuli tend to reverse this. However, MMP activity cannot be directly measured in VSMC con-
ditioned media (50) because of an excess of TIMPs (142). This may imply that MMPs produced from VSMC alone are unable to remodel the ECM. On the other hand, MMP activity can be detected in VSMC in vascular tissues by in situ zymography, a powerful methodology whereby frozen histological sections are coated with and allowed to degrade MMP substrates that are subsequently visualized (90). This suggests that MMP activity may not be completely abolished by TIMP binding but could be confined temporally, or spatially, to the pericellular region surrounding VSMC (309).

B. EC

As in VSMC, MMP-2 expression is constitutive in EC (106) and further upregulated by ROS (120). However, induction of the MMPs is less obviously staged in EC. MMP-1 and -2 are upregulated by high glucose concentrations (67), implying links to both basement membrane and interstitial matrix degradation in diabetic vascular disease. Similarly, concomitant upregulation of MMP-1, -3, -8, and -9 occurs in response to TNF-α and IL-1α (106, 114) and on coculture with macrophages (117, 314). CD40 ligation also upregulates a broad spectrum of MMPs in EC, including MMP-1, -3, -8, -9, and -11 (171, 241). Inflammatory and immune mechanisms therefore seem to be the main drivers of matrix remodeling by EC, with no obvious dissociation of basement membrane and interstitial matrix degradation. Particularly relevant to atherosclerosis, oxidized LDL increases MMP-1 and -3 expression (119, 155) via the Lox-1 receptor and activation of protein kinase C-β. Oscillatory but not laminar flow induced a large increase in MMP-9 transcription and activation in a murine endothelial cell line (174), and this could have some bearing on the localization of atherosclerotic plaques. Growth in three-dimensional collagen (103) and cyclic strain (303) upregulate MT1-MMP (MMP-14), while increased shear stress downregulates MMP-14 (307). These phenomena are relevant to microvascular endothelium and angiogenesis (212).

EC express TIMP-1 and TIMP-2 constitutively (106), and this is not increased by oxidized LDL (155). TIMP-1 is further upregulated in response to CD40 ligation (171). MMP activity is nevertheless directly detectable in conditioned media from EC (120), which implies that EC are potentially more active in matrix remodeling than VSMC.

C. Macrophages

A similar multistage upregulation of MMP genes as that found in VSMC (Fig. 4) is clearly evident in macrophages. At least in human monocyte-macrophages, MMP-9 appears to be the most abundant gelatinase. Contact of peripheral blood monocytes with EC rapidly upregulates MMP-9, via a 30-kDa soluble mediator (7). Adhesion to matrix components including collagen, fi-
bronectin, and tenascin-C also upregulates macrophage MMP-9 secretion (91, 285, 290). Integrin-dependent adhesion to collagen acts synergistically with P-selectin-dependent adhesion to platelets to increase MMP-9 secretion by both transcriptional and translational mechanisms (91). MMP-9 expression is further stimulated to a small extent by soluble mediators IL-18 and TNF-α (202, 233) and also by oxidized LDL (301). Proatherogenic cytokines and growth factors, including IL-1β, TNF-α, macrophage colony stimulating factor (M-CSF) and PDGF, upregulate macrophage MT1-MMP (MMP-14) (221) and MT3-MMP (MMP-16) expression (280) and could therefore activate MMP-2 secreted constitutively from VSMC or EC. Because MMP-1 and -3 are not upregulated by cytokines in macrophages (233), the increased production of MMP-9 and activation of MMP-2 could selectively mediate basement membrane turnover. Cytokines and growth factors also increase macrophage MMP-12 (80), which aids monocyte migration into tissues (250). Immune activation via CD40 ligand upregulates MMPs-1, -3, -8, -9, and -11 (114, 170, 172, 176, 241, 300), which have the ability to remodel interstitial matrix also. MMP-1 secretion is also upregulated by contact with EC (117).

While these mechanisms are no doubt relevant to atherosclerosis, they probably do not completely account for the increase in MMP secretion observed from foam cell macrophages. Isolated, lipid-laden, macrophages from aortic atheroma or from subcutaneous granulomas in cholesterol-fed rabbits continue to express MMPs-1, -3, -9, and -13 for long periods in culture (50, 89). The upregulation of MMPs-1 and -3 depends on engorgement with lipid because it does not occur in nonfoamy alveolar macrophages from lipid-fed rabbits (89) or granuloma macrophages from chow-fed rabbits (50). The mechanism is apparently complex because simple addition of ox-LDL to human macrophages does not upregulate MMPs-1 and -3 (191). This implies that foam cell formation somehow activates the intrinsic transduction pathways leading to MMP secretion or constitutive production of autocrine factors. Consistent with the first possibility, treatment with the scavenger N-acetyl-L-cysteine decreases MMP-9 expression, which implies that ROS are involved (85), and foam cell macrophages show constitutive activation of the redox-sensitive transcription factor NF-κB (50). Consistent with the second possibility, preliminary work from our laboratory suggests that upregulation of MMP-1 but not MMP-3 from foam cells is in part mediated by a soluble autocrine factor unrelated to CD40 ligand (22).

Human macrophages and rabbit foam cells secrete TIMP-1, TIMP-2, and TIMP-3 constitutively (50, 76, 168), while ox-LDL treatment decreases TIMP-1 secretion, partly at least through autocrine action of IL-8 (191, 301). Despite TIMP secretion, MMP activity is measurable in macrophage and foam cell conditioned media (50, 168), and macrophage-conditioned media degrade collagen from isolated plaque caps in vitro (245). MMPs secreted by macrophages could also function as activators of MMPs derived from other cell types including VSMC.

D. T Lymphocytes

T lymphocytes in atherosclerotic plaques stain positive for MMPs-1, -2, -3, and -9 by immunocytochemistry (90), but relatively few studies of their MMP secretion have appeared. Interaction with fibronectin through α4β1-integrins upregulates MMP-2 and -9, while ligation of vascular cell adhesion molecule (VCAM)-1 selectively increases MMP-2 (302). The resulting conditioned media contain MMP activity (302), and these proteases may therefore have a role in transmigration of T cells into sites of inflammation. MMP-2 is also increased in response to the cytokine IL-2 (153).

E. Mast Cells

Mast cells constitutively secrete MMP-2 and upregulate MMP-9 secretion in response to TNF-α kit ligand (KL, stem cell factor) and contact with activated T cells (17, 78). Induction of the MMP-9 gene and secretion of MMP-9 protein appear to be separately regulated by T-cell contact (17). T-cell contact also increases TIMP-1 secretion (17); the net effect on MMP activity was not studied.

F. Adventitial Fibroblasts

Overall the pattern of constitutive MMP-2 secretion and synergistic upregulation by cytokines and growth factors of MMP-1, -3, and -9 seems very similar in fibroblasts and VSMC (33, 35). However, the levels of MMP-2 and -9 secretion from pig coronary adventitial fibroblasts are significantly greater than from VSMC (247). Even more strikingly, the levels of TIMP-1 and -2 expression are more than 10-fold less in the fibroblasts (247). Fibroblasts may therefore have a greater propensity to generate MMP activity, which could give them an advantage over VSMC when migrating into areas of vascular injury (247).

G. Transcriptional Control Mechanisms

The basis of the staged induction of different MMP genes by stretch, injury, inflammation, and immune activation can be partially understood from the known structures of their proximal promoter regions (51). Among the secreted MMPs, MMPs-2 and -11 are unusual in not having a TATA or CAAT box, which implies that they are largely constitutively expressed, housekeeping genes. Nevertheless, the transcription of MMP-2 can be upregulated by two far upstream enhancer regions that respond to Y-box
protein-1, p53, activator protein-2, and GATA-2 transcription factors (105). The promoters of many secreted MMPs have a TATA box and proximal activator protein-1 (AP-1) binding site, which is essential for upregulation (reviewed in Refs. 21, 80). There are also multiple AP-1 and polyoma virus enhancer-A-binding protein-3 (PEA-3)/ets (PEA-3/ets) sites further upstream. Individual MMP gene promoters contain other unique transcription factor binding sites. For example, a STAT-1 binding element (SBE) in MMP-1, stromelysin PDGF response element (SPRE) in MMP-3, Tcf/LEF in MMP-7, and nuclear factor κ-B (NF-κB) sites in MMP-9 promoters, respectively, probably contribute to their staged induction (reviewed in Ref. 51). These transcription factors apparently act cooperatively with each other and the core AP-1 site, which in part accounts for the synergistic effects of agonists (34, 75). Oddly, activation of the NF-κB transcription factor is required for induction of MMP-1 and -3 in VSMC (34) and macrophages (50), even though their proximal promoters lack functional sites for binding NF-κB. One explanation for this could be induction through NF-κB-dependent pathways of other unknown transcription factors, although this has not been demonstrated. The MMP-9 promoter also has a c-Myc binding site, which participates in responses to oscillatory flow (174). The promoter region of MT1-MMP (MMP-14) is distinctly different from those of the secreted MMPs, since it lacks a TATA box and contains a proximal Sp-1 rather than AP-1 site (165). Activity of the MMP-14 promoter appears to be upregulated either by Sp-1 phosphorylation or by its displacement with the Egr-1 transcription factor (303, 307).

VII. PARTICIPATION OF MATRIX METALLOPROTEINASES AND TISSUE INHIBITORS OF METALLOPROTEINASE IN INTIMAL THICKENING BY REGULATION OF CELL MIGRATION AND PROLIFERATION

A. Methodologies

Well-established methodologies have been developed to measure intimal expansion and its component processes. Endothelium-denuded medial tissue can be stripped from the adventitia and VSMC isolated by outgrowth from explants (187) or by digestion with collagenase (43). Such cells progressively lose their normal complement of contractile and cytoskeletal proteins and increase their content of synthetic organelles, a process known as phenotypic modulation (273). In fact, a variety of different VSMC phenotypes have been isolated from animal and human arteries and veins (108). Some are fibroblast-like in shape and grow over each other in a typical hill and valley formation, while others are epithelioid and grow as monolayers. EC can be selectively removed from vessels by brief collagenase digestion; depending on the culture conditions, they grow as monolayers or as capillary-like tubes (212).

Freshly isolated VSMC can be allowed to undergo contractile to synthetic phenotype modulation under controlled tissue culture conditions, especially when grown on the interstitial matrix component fibronectin (111, 112). Phenotypic change can be delayed by culture on the basement membrane component laminin (110), while addition of heparin, TGF-β, and, at least for human cells, growth at high density tends to reverse the phenotypic change (100). Growth of human SMC at high density can give models for other pathological features including calcification (216).

Migration of isolated VSMC and EC can be measured in wounded monolayers (161) or by seeding isolated cells onto thin or thick layers of collagen and other synthetic matrices, such as matrigel (82, 184). Measuring the rate of emigration of cells from explants (256), especially in the presence of hydroxyurea to inhibit cell proliferation (136), allows studies of the effects of native ECM barriers. Cell proliferation is commonly measured by cell counting, incorporation of DNA precursors, measurement of cell cycle specific proteins, and flow cytometry. Death is measured by cell counting, dye penetration, nuclear shape, and a variety of assays selective for apoptosis, such as caspase activation and DNA end-labeling (206, 286).

Organ culture (140, 259) gives a simple in vitro assay for mechanistic studies of neointima formation and is one of the few tools that can be directly used with human tissue. Pulsed or continuous labeling of the cultures with DNA precursors can be used to measure proliferation. However, some VSMC that migrate to the neointima do not subsequently proliferate and can be used as a measure of migration alone (92, 93, 259). In vivo, rat, rabbit, and pig models of arterial balloon injury have been widely used. These show immediate endothelial denudation, apoptosis of medial VSMC (175), and in some cases rupture of the media (246). Early proliferation of VSMC follows in the media. Migration of VSMC (57) (and fibroblasts, Ref. 246) to the neointima and their proliferation occur later. Intimal VSMC lay down excess ECM, which contributes to thickening (57). As in the organ cultures, some VSMC that migrate to the neointima do not subsequently proliferate and can be used as a measure of migration alone (59). However, more commonly, migration is quantified by measuring the number of cells that appear in the neointima at an early time point, e.g., 4 days (124). Neointima...
formation in balloon injury models is self-limiting, in part by endothelial regrowth, and may actually regress by enhanced apoptosis and compaction of the ECM (57). Investigators have usually studied the response to injury of normal blood vessels, although in a minority of cases injury has been superimposed on an experimental plaque (54). Even so, the relevance of any of these models to clinical angioplasty in humans has been severely questioned. First, the ready proliferation of VSMC in the animal models does not seem to be observed in human restenotic lesions (203). Second, clinical restenosis may be more closely related to shrinking of the vessel wall (negative remodeling) rather than to neointima formation (71, 188). Recently, animal models of stent deployment in normal arteries have been developed to study the phenomenon of in-stent restenosis (81, 138). These models show foreign body reaction to the stent and localized intimal growth that mirrors the human situation. However, these models do not usually consider the important contribution of existing atherosclerosis to subsequent human in-stent restenosis (79). Rodent and large mammal models of vein grafting have also been described. We recently reviewed the comparative merits of these models, pointing out that only the large animal models reproduce the size and therefore the mechanical changes of human bypass grafting (292). However, induction of vein graft atherosclerosis is much easier in rabbit and mouse models.

A particular cautionary note is necessary when considering the available models in mice, since so much recent research has sought to harness the power of their genetic manipulation. Because of their relative size, mice are likely to have lesions less than 1,000 times smaller in terms of mass and volume compared with human lesions (62). Vessel diameters are correspondingly smaller, and the thickness may be just a few cell layers. In addition, normal mice carry most of their plasma cholesterol as high-density lipoprotein (HDL), not LDL. Species-related differences in protein expression (importantly MMP-1, see below) also make extrapolation from mouse to human a risky enterprise.

Injury to healthy mouse carotid artery has been conducted using filament loops, guide wires, dessication, adventitial cuffing, and flow cessation, although none of these is the mechanical equivalent of clinical balloon angioplasty. An ingenious mouse vena cava into carotid artery vein-grafting model was developed by Xu and colleagues (315). However, this may differ crucially from vein grafting in humans because the mouse vena cava is so thin walled that all of its cells undergo apoptosis under the strain of arterial pressure (315), and the graft becomes repopulated with cells from the graft and the host (118). The extent to which similar processes occur in the much thicker walled human vein grafts is uncertain.

B. Patterns of MMP Expression and Activity in Humans and in Animal Models of Neointima Formation

Life-threatening complications early after angioplasty are thankfully rare. Hence, there have been few histological and biochemical studies of MMPs in human restenotic lesions and none in the crucial first few days and weeks after injury. Nikkari et al. (200) observed increased collagen transcripts in restenotic lesions 6–18 mo after angioplasty. Levels of MMP-1 and TIMP-1 were reduced compared with normal tissue, which implies a balance towards fibrosis at these relatively late time points. In human saphenous veins, upregulation and activation of gelatinases (MMP-2 and -9) occurs within hours after injury caused by surgical preparation and continues during neointima formation in organ cultures (95). Active gelatinases are detected in the media and neointima by in situ zymography (95, 142), even though TIMP-1 and TIMP-2 levels are also increased (142). Upregulation of MMP-1 and MMP-3 is much less evident in human saphenous vein (132). Given the paucity of direct human pathological observations, other approaches including genetic studies are appropriate. A single genetic study identified a functional A-82G polymorphism in the MMP-12 promoter, which affected the AP-1 binding site (133). The A allele increased promoter activity and favored luminal narrowing in a small group of 71 diabetic patients, who underwent angioplasty and stenting.

There is abundant evidence of gelatinase upregulation in animal models of neointima formation. Induction of MMP-9 and activation of MMP-2 occur rapidly after balloon injury to rat (25, 311), pig (257), baboon (136), rabbit (12), and mouse (98, 160) arteries. In the pig and baboon models, induction of MMP-9 and activation of MMP-2 occurred together (136, 257), but MMP-2 activation was delayed in the rat and mouse studies (25, 98, 160, 311), perhaps pointing to a preeminent role for MMP-9 in small rodents (see below). Activation of MMP-2 is associated with upregulation of MMP-14 and -16 expression in balloon-injured rat carotid arteries, implying that activation of MMP-2 by these MT-MMPs is feasible (251). Levels of MMP-1, -3, -12, and -13 were also increased after femoral artery injury in mice (160). TIMP-1 and TIMP-2 expression are either not changed or increased after balloon injury (109, 287), while levels of TIMP-3 (unpublished observations) and TIMP-4 are clearly increased (73). Increased expression and activation of MMP-2 and -9 was also observed in other in vivo models of neointima formation, namely, pig vein grafts (258) and the rabbit iliac artery after angioplasty and stenting (81).

Hence the picture of early and possibly selective upregulation of gelatinases is highly consistent across a variety of models of neointima formation, irrespective of the provoking stimulus. Levels of TIMPs are either main-
tained constant or increased so that changes in TIMPs do not amplify but tend to counteract the effects of MMP upregulation. What then are the mechanisms responsible for MMP upregulation? First, this could be the direct result of injury because MMP-1 and -3 upregulation occur in injured monolayers of isolated VSMC (125). MMP-9 induction and MMP-2 activation also occur in human saphenous veins injured by hydrostatic distension ex vivo even before culturing (95). Increased intraluminal pressure produces a similar response in pig carotid arteries too (55), although this may be related to stretch per se rather than injury. Inflammation is another possible cause of MMP upregulation, although neointima formation in organ cultures of human saphenous vein proceeds in the presence of only scattered resident macrophages (60). Balloon injury to the rat carotid artery also provokes little inflammatory response (277). However, balloon injury to other arteries (270), stent implantation (138), and vein grafting (291) provoke a vigorous inflammatory response. As expected, there is initial neutrophil infiltration followed by more sustained macrophage deposition (79, 291). Lymphocytes may also be present especially after stent implantation (138). Hence, there is potential for MMP induction by soluble inflammatory mediators and CD40 ligation, as identified from in vitro studies (see above).

C. Effects of MMP Overexpression and TIMP Knockout

Clowes and colleagues (184) prepared rat VSMC stably transfected with the MMP-9 gene under a tetracycline-regulated promoter. They found that MMP-9 overexpression had no effect on VSMC proliferation but promoted migration through matrigel and three-dimensional collagen (161). Migration through matrigel and three-dimensional collagen had no effect on VSMC proliferation but promoted regulated promoter. They found that MMP-9 overexpressed with the MMP-9 gene under a tetracycline-activated promoter was valuable to verify these results with TIMP gene transfer. Seeding of cells stably overexpressing TIMP-1 reduced neointima formation after either plain balloon angioplasty or angioplasty with stenting (54). Given that high concentrations of synthetic inhibitors might have effects other than on activity of MMPs, for example on mitogen-activated protein kinases (167), it was valuable to verify these results with TIMP gene transfer. Seeding of cells stably overexpressing TIMP-1 reduced neointima formation after balloon injury (82), as did adenovirus-mediated gene transfer of TIMP-1 or TIMP-2 (53, 72). TIMP-1, -2, and -3 gene transfer into human saphenous vein prevented neointima formation at the distal anastomoses of polytetrafluoroethylene arteriovenous shunts in pigs after 4 wk (230); longer follow-up is clearly warranted to see whether the benefit is maintained.

D. Effects of MMP Inhibition and Knockout

Two structurally unrelated synthetic MMP inhibitors inhibited both proliferation and emigration of VSMC from rabbit aortic explant cultures (256). The effects on migration but not proliferation were also found in baboon saphenous artery explants (136). Studies with synthetic MMP inhibitors in the rat carotid balloon injury model showed a consistent effect on early SMC migration (23–25, 121, 312). Two of the five studies found an effect also on the first wave of medial (121, 312) and two on the second wave of intimal (23, 121) VSMC proliferation. However, except in one study (121), MMP inhibitors had no effect on the final intimal size (23, 24, 312), possibly because of late catch-up proliferation in the neointima. A likely explanation for this is redundancy between the MMP and other plasminogen-mediated proteolytic pathways (136). A study of balloon angioplasty to the atherosclerotic iliac arteries of Yucatan micropigs also showed no effect of MMP inhibition on intimal growth (68). However, MMP inhibition significantly antagonized constrictive remodeling and therefore led to a decrease in late lumen loss (68). In a recent, thorough preclinical study in atherosclerotic primates, a broad spectrum MMP inhibitor was infused into animals for 28 days to achieve inhibitory plasma concentrations. MMP inhibitor treatment failed to reduce neointima formation after either plain balloon angioplasty or angioplasty with stenting (54). In contrast to the study in pigs (68), no effect on constrictive remodeling was observed. In contrast to these largely negative results, it was very recently reported that a broad spectrum MMP inhibitor reduced neointima formation at the distal anastomoses of polytetrafluoroethylene arteriovenous shunts in pigs after 4 wk (230); longer follow-up is clearly warranted to see whether the benefit is maintained.
to its effects on vein grafts. From these studies, catch up growth does not seem to compensate for the effects on neointima formation of TIMP gene transfer in contrast to those of synthetic MMP inhibitors, at least over the 2- to 4-wk time periods studied. The reasons for this are obscure but could involve any of the several effects of TIMPs that are unrelated to MMP inhibition (15, 218).

While synthetic inhibitors and TIMP gene transfer target a broad spectrum of MMPs, knockout studies have the potential to elucidate the role of individual MMPs. Knockout of MMP-11, the unusual MMP that degrades serum proteinase inhibitors (see Table 1), increases neointima formation after injury in mice (162). MMP-11 knockout was associated with a paradoxical increase in elastolysis and increased cellularity of the neointima rather than matrix accumulation (162). Two groups independently studied the effect of MMP-9 knockout on responses to filament loop injury (56) or arterial occlusion (86) in different background strains of mice. In wild-type mice, both groups observed early upregulation of MMP-9 and later activation of MMP-2 that was apparently unrelated to inflammation. MMP-9 knockout impaired neointima formation by inhibiting VSMC migration. One of the groups (56) also observed delayed inhibition of VSMC proliferation. Interestingly, MMP-9 knockout caused excess collagen accumulation and impaired outward remodeling in the occlusion model (86). More recently, two groups also found that knockout of the other gelatinase, MMP-2, also decreased neointima formation in the carotid ligation model and that this was associated with decreased VSMC migration in vitro (128, 144). Hence, MMP-2 and -9 appear to serve complementary rather than redundant functions in neointima formation, despite similar structure and substrate specificity and the compensatory upregulation of MMP-9 and downregulation of TIMP-1 expression evident in MMP-2 knockouts in one of the studies (144). The ability of MMP-9 but not MMP-2 to participate in CD44-mediated matrix attachment of VSMC and to organize collagen fibrils may account in part for the unique contribution of this MMP (128).

E. Putative Mechanisms

MMPs could facilitate VSMC migration by a variety of mechanisms. First, they could simply relieve the direct binding between basement membrane components and cell surface integrins that physically restrict movement. Second, MMPs could aid invasion of already freely moving VSMC by degrading the physical ECM barriers they encounter. Consistent with this, addition or gene transfer of MMPs promotes (184) and gene transfer of TIMPs inhibits (16) invasion of collagenase-isolated VSMC through synthetic matrix. Third, cleavage by MMPs could expose adhesion sites on matrix molecules over which VSMC could then gain traction. For example, Gavrilovic's group showed that cleavage of collagen I into one-fourth and three-fourth length fragments using MMP-13 produced a substrate upon which PDGF stimulated VSMC could migrate more rapidly than on intact collagen I (266). Moreover, adhesion and migration on cleaved collagen was mediated by newly induced α5β1-integrins, while migration on native collagen I was mediated by constitutive α2β1-integrins. This observation exemplifies a fourth mechanism, namely, that MMPs might modify the cell-to-matrix interactions of VSMC, which in turn changes their behavior by phenotypic modulation. Modulation of contractile VSMC towards a more synthetic phenotype accompanies neointima formation (42, 49, 274). During phenotypic modulation, basement membrane components, for example, laminin, are downregulated, while hyaluronic acid production (237, 293) and interstitial matrix components, monomeric type I collagen, elastin, and fibronec tin are greatly increased (9, 273, 275) (Fig. 5). Matrix remodeling is of key importance because laminin...
retards or partially reverses phenotypic modulation, while fibronectin promotes it (110, 111). Hence, MMPs could promote phenotypic modulation by relieving interactions with inhibitory components or promoting interactions with modified stimulatory components. These effects of matrix proteins on migration are likely to be mediated by concomitant changes in expression of their cell surface integrin receptors (189) (Fig. 5).

One mechanism by which proteases might influence intimal growth is by the release of sequestered cytokines, such as FGF-2 and TGF-β (264). MMPs might also degrade matrix proteins with inhibitory effects on VSMC proliferation. For example, heparan sulfate proteoglycan (HSPG) components of the basement membrane, such as syndecans and perlecan, inhibit VSMC proliferation in culture and in vivo (29, 139). Hence, the ability of MMPs to degrade core proteins of HSPGs could underlie their effect on VSMC proliferation. Alternatively, basement membrane remodeling could permit new contacts with interstitial matrix components that promote cell cycle progression. Contractile VSMC in arteries exhibit very low rates of proliferation, most likely because they fail to down-regulate cyclin-dependent kinase inhibitors (123, 271, 272). Components of the ECM of normal arteries, e.g., laminin and polymerized collagen, do not support down-regulation of cyclin-dependent kinase inhibitors or proliferation of isolated VSMC (141). On the other hand, components of the remodeling matrix such as monomeric collagen and fibronectin support G1 cell cycle progression and VSMC proliferation (141, 244). Again, these actions of matrix proteins are believed to be mediated by binding to appropriate integrin receptors (244).

Few studies have directly measured the influence of MMPs on matrix protein turnover in blood vessels. Strauss et al. (265) found that MMP inhibition decreased collagen breakdown after balloon injury to rabbit femoral arteries, as might be expected. However, collagen synthesis was also inhibited, presumably because MMP inhibition prevented VSMC transforming into the synthetic phenotype. The net effect on neointima formation was neutral (265). A reversible disappearance of basement membranes around VSMC and an increase in fibronectin synthesis were demonstrated during neointima formation in balloon-injured rat carotid arteries (275). These changes track the changes in MMP expression established in other studies (25, 311), but the influence of MMP inhibition on ECM proteins was not tested directly. To achieve this, we investigated the effect of TIMP-1 and -3 gene transfer on expression of basement membranes in human saphenous vein undergoing neointima formation in organ culture (1). Medial VSMC were normally surrounded by basement membranes, but a proportion became negative during culture. All VSMC that migrated into the neointima lacked basement membranes. TIMPs-1 and -3 reduced the numbers of medial VSMC lacking basement membranes and inhibited migration into the neointima. This implies that MMP-mediated loss of basement membrane is essential for medial to intimal migration of VSMC. Proliferating VSMC were more likely to be negative for basement membranes than nonproliferating VSMC. On the other hand, 30 – 40% of proliferating medial VSMC retained some basement membrane, which shows that complete basement membrane degradation is not necessary for VSMC proliferation. Furthermore, TIMPs did not affect either basement membrane degradation in proliferating VSMC or the rate of medial VSMC proliferation. Hence, MMPs are not essential for basement membrane degradation in proliferating VSMC, presumably because of redundancy with other proteases. These conclusions are summarized in Figure 6.

Many of the nonmatrix substrates of MMPs (see Refs. 264, 283 for listings) may also be relevant to neointima formation and plaque instability, but as yet, there have been few direct studies in VSMC. The arguments seem particularly clear for MMP-11, however, which is only weakly active against matrix proteins. Proteolysis of insulin-like growth factor binding protein (IGFBP)-1 by MMP-11 (177) and IGFBP-3 and -5 by MMP-1, -2, and -3 (83) could have implications both for phenotypic modulation (110) and survival (210) of VSMC. The ability of the gelatinases, MMP-2 and -9, to activate TGF-β (306) could feed forward to promote matrix protein synthesis after...
basement membrane remodeling, although this has not been shown in vascular tissue. The potential of at least seven MMPs to activate TNF-α (264) invites the speculation that they further contribute to the inflammatory milieu in injured or atherosclerotic vessels. Direct experiments to test these ideas would be welcome. In fibrosarcoma cells, MT1-MMP (MMP-14) can be involved as well as furinlike proprotein convertases in the processing of αv-integrin subunits, and this affects attachment to type I collagen (14, 222). However, the thiol proprotein convertase PC5 rather than MMP-14 appears to be responsible for processing of αv-integrin subunits in rat VSMC (262). Expression of the MT-MMPs, MMP-14 and -16 has recently been linked to degradation of focal adhesion kinase, cell rounding, decreased adhesion, and increased migration in baboon aortic VSMC (252), although this occurred independently of changes in integrin levels (252). A synthetic metalloproteinase inhibitor concomitantly reversed all these events (252). However, no evidence was presented that focal adhesion kinase is a direct substrate for MT-MMPs, the topography of which makes it unlikely. The identity of the matrix or nonmatrix substrates responsible for this interesting phenomenon therefore remains obscure.

Using human VSMC, George’s group (277) recently reported that MMP-mediated cell surface shedding of N-cadherin occurs after serum or PDGF stimulation (Fig. 7). This releases β-catenin, which translocates to the nucleus and participates in stimulating proliferation (277). Finally, the evidence that MMP-9 can promote matrix attachment of VSMC through binding to CD44 and that this promotes collagen assembly demonstrates the possibility of noncatalytic mechanisms by which MMPs could influence intimal thickening (128).

F. Conclusions

The evidence for MMP-9 upregulation and MMP-2 activation early during neointima formation after vascular injury is overwhelming. Evidence for upregulation of other classes of MMPs is less abundant. Rather than decreasing to exaggerate the effect of MMP induction, TIMPs are concomitantly upregulated, presumably to prevent excessive matrix degradation. From studies with synthetic MMP inhibitors or TIMP gene transfer, MMPs are proven to remodel the VSMC basement membrane, and this is required for VSMC migration and phenotypic modulation. Effects of MMP inhibition on VSMC proliferation appear restricted to certain models and early time points, probably because of redundancy with other proteases. As a result, there is catch up growth so that MMP inhibition does not lead to a change in final intimal size. MMPs are also expressed in fibroblasts and may therefore play a part in adventitial remodeling or migration of myofibroblasts into the intima.

VIII. ROLE OF MATRIX METALLOPROTEINASES IN ATHEROSCLEROTIC PLAQUE DESTABILIZATION

A. Methodologies

In humans, correlative histological studies have been the main investigational approach. Tissue from aortic, carotid, and to a lesser extent coronary plaques at all stages of their development, including ruptured plaques, are available from post mortem, endarterectomy, and atherectomy specimens. Human studies correlating presence of unstable coronary disease with serum MMP measurements (32) or MMP genotypes (313) have recently emerged. Rabbit, pig, and primate models of diet-induced atherosclerosis have also been used. At early times, the animal lesions resemble human fatty streaks, although fibrous cap development does occur within weeks, especially after episodic removal of cholesterol-rich diet (2). More complex lesions occur spontaneously in rabbit (249) and pig (96) models, albeit slowly, and so have been less studied. So-called humanlike atherosclerotic plaques occur in the aortic root, thoracic aorta, and brachiocephalic arteries of susceptible strains and genetically modified mice (196, 207, 227), although caveats have been raised (see above) about their small size and the altered lipid metabolism in mice compared with hu-

**Fig. 7.** MMP-dependent shedding of N-cadherin from human VSMC. Expression of N-cadherin by Western blotting in control human saphenous vein VSMC is increased by treatment with the MMP inhibitor BB-94 or after adenovirus-mediated transfer of tissue inhibitor of metalloproteinase (TIMP)-1 or TIMP-2. The same treatments increase N-cadherin in the membrane (M) but not the cytosolic (C) or nuclear (N) fractions of the cells. [From Uglow et al. (277).]
There is also evidence of coronary atherosclerosis leading to myocardial infarction in mice, although probably through vasoconstriction rather than plaque rupture (41). Myocardial infarction apparently unrelated to plaque rupture also occurs in a strain of Watanabe rabbits (249).

Although two reviews (62, 225) concluded that there is no universally accepted animal model for spontaneous plaque rupture, substantial progress has been made recently using mice. Calara et al. (40) observed rare plaque instability in the aortas and coronary arteries of ApoE and LDL receptor double knockout mice fed cholesterol for 1 year. Rosenfeld et al. (227) and Johnson and Jackson (130) described a higher frequency of intraplaque hemorrhage in the brachiocephalic arteries of fat-fed ApoE knockout mice. Discontinuity of the very thin (2 μm) fibrous cap suggested that these lesions result from plaque ruptures (Fig. 8), in some degree analogous to those in humans (130). Plaque rupture occurred both in mice that died spontaneously and in those culled according to protocol (296), which implies that not all ruptures were fatal. Moreover, just as with lesions in humans, many of the mouse plaques had buried fibrous caps, consistent with previous silent plaque ruptures. Models of induced plaque rupture have also been described (62, 225), but their use is probably restricted to investigating plaque cap mechanical properties or subsequent thrombosis.

In the absence of widely accepted models of plaque rupture, most work has used surrogate end points in rabbit or mouse models of atherosclerosis progression. Lesion size measured by standardized histology of the aortic root (207), or defined points in the aorta (255) and lesion extension measured by lipophilic staining of the aorta en face is popular. Multiple anatomic sites have occasionally been investigated. Measurement of plaque composition in relation to known features of human vulnerable plaques has been conducted. According to this, a relatively large proportion of the plaque occupied by lipid core or monocytes, a low proportion occupied by smooth muscle cells and a low content of collagen is taken as indicative of plaque vulnerability. Most interestingly, a recent study (296) compared occurrence of ruptures in mice brachiocephalic arteries, defined by discontinuity of the fibrous cap and thrombus penetrating into the lipid core, with several of these surrogate markers. Consistent with clinical data, ruptured plaques were larger and had a larger proportion of the plaque occupied by lipid core, fewer VSMC, and a thinner fibrous cap. They also had more buried plaque caps, consistent with more previously silent plaque ruptures.

Because of the similar inflammatory origin of aneurysms and unstable plaques (see sect. II), results from models of atherosclerotic aneurysm formation are also relevant. The ApoE knockout mouse shows frequent breaches of the medial elastic fibers that can be interpreted as microaneurysms (47). Galis and co-workers (122) recently showed that expansive remodeling after carotid artery ligation is greatly increased in ApoE knockout compared with wild-type mice. This may therefore also serve as a model of atherosclerotic aneurysm formation. Other mouse models of aortic aneurysm not linked to atherosclerosis use elastase (217) or calcium chloride (166) treatment to provoke the initial inflammatory insult.
Another model in rats involved xenotransplantation of guinea pig aortas (6).

B. Patterns of MMP Expression and Activity in Human Pathological Tissues and Experimental Models of Atherosclerosis

A landmark study by Henney et al. (113) demonstrated upregulation in atherosclerotic plaques relative to normal artery of MMP-3 mRNA, and its location within macrophages and VSMC. Galis in Libby’s group (90) performed a more exhaustive study showing MMP-1, -3, and -9 protein in macrophages, VSMC, lymphocytes, and EC especially at the vulnerable shoulder regions of atherosclerotic plaques. Moreover, the authors developed the in situ zymography technique to localize MMP activity and showed that this was present in plaques but not normal media (90). Subsequent work confirmed and extended these results. Elevated levels of MMP-1 (90, 200, 201), MMP-2 (156), MMP-3 (90, 113), MMP-7 (104), MMP-9 (38, 90), MMP-11 (241), MMP-12 (104), MMP-13 (268), MMP-14 (MT1-MMP) (221), and MMP-16 (280) are all found in human atherosclerotic plaques, especially at the macrophage-rich shoulder regions. Enhanced MMP-1 overexpression is located with areas of increased circumferential tensile stress in the fibrous cap (148) where ruptures are likely to occur. MMP-7 and lesser amounts of MMP-12 are confined to the borders between the lipid core and the macrophage-rich shoulder regions (104). MMP expression is prominent in macrophages but also present in VSMC, lymphocytes, and EC (38, 90, 104, 221, 268, 280). TIMP-1 levels are unchanged (90) or elevated (200) in atherosclerotic plaques. TIMP-2 is also abundant in plaques (221), and TIMP-3 is clearly increased because it is a product of inflammatory macrophages (76).

Henney’s group was also the first study to relate polymorphisms in any of the MMP genes to the incidence or severity of coronary artery disease. They reported a 5A6A polymorphism in the MMP-3 promoter for which the 6A allele was associated with greater progression of coronary disease in a small cohort of patients (305). They later found a C-1562T polymorphism in the MMP-9 gene (313). The T allele resulted in greater promoter activity (313) and was subsequently shown to influence MMP serum levels (32). The T allele was associated with increased disease severity (192, 313), although it had no impact on prognosis in patients with existing coronary disease (32). On the other hand, increased MMP-9 serum levels were strongly associated with other markers of inflammation and were significantly associated with cardiovascular death (32). These results support a role for MMP-9 as a marker or etiological factor in atherosclerosis progression and possibly instability.

Upregulation of MMPs-1, -2, -3, and -9 is readily detected in models of lipid-induced atherosclerosis in rabbits (2, 310), and MMPs-3, -11, -12, and -13 are also upregulated in mouse plaques (126, 231, 241). Upregulation of MMPs is an early event in fatty streak formation in rabbits and mice and is therefore associated with plaques at all stages, not just in the late events of plaque instability. In cholesterol-fed rabbits, the levels of TIMP-1 and TIMP-2 clearly rise in plaques, and this partly compensates for the increase in MMP activity (309). In particular, it was suggested that this confines MMP activity to the pericellular region, stabilizes the base of the lesions, and may prevent plaque penetration into the media (i.e., aneurysm) (309). Similar conclusions can be reached for TIMP-3 (C. Aguilera, A. Zaltsman, and A. C. Newby, unpublished observations).

Further correlative studies have attempted to establish a link between MMP overexpression and destruction of individual matrix components. For example, MMP-7 is apparently the most active versican-degrading enzyme in human carotid artery plaques (104). On the other hand, and perhaps most convincingly, Sukhova et al. (268) showed that MMP-1 and MMP-13 in human lipid-rich atherosclerotic plaques are closely colocalized with neoepitopes of cleaved collagen (Fig. 9) (268).

Other studies have related MMP levels to clinical or histological features associated with plaque instability. Most simply, Schonbeck et al. (241) found that MMP-11 immunoreactivity is confined to advanced atherosclerotic plaques, not fatty streaks. In carotid endarterectomy specimens, Nipparki et al. (200) noted that positivity for MMP-1 correlates with the percentage of the lipid core occupied with hemorrhage. However, there was no correlation between MMP-1 expression and presence of rupture or clinical symptoms of instability. Loftus et al. (164) found no difference in MMP-1, -2, and -3 or TIMP-1 and -2 levels in carotid endarterectomy tissue from patients that had recent (<1 mo) clinical evidence of instability, even though this group had a greater prevalence of ruptured plaques, intraplaque hemorrhage, and distal embolization. In contrast, the same study showed a significant two- to fourfold increase in MMP-9 levels in the most recently symptomatic plaques (164). A small atherectomy study also suggested that MMP-9 expression is more prevalent in patients with unstable versus stable coronary disease (38). Finally, in 25 patients with unstable and 10 with stable angina, intense MMP-3 expression was found selectively in outwardly remodeled plaques (239), but there was no relationship to clinical presentation. From these studies the clearest evidence is for a positive relationship between MMP-9 expression and plaque instability. However, these case-control studies cannot distinguish whether differences in the levels of MMPs were a cause or an effect of plaque instability. Moreover, increased MMP-9 levels
could be an epiphenomenon, simply reflecting the increased prevalence of macrophages in unstable plaques.

Upregulation of MMPs-1, -3, -9, -12, -13, and -14 is also seen in association with inflammatory matrix destruction in human aortic aneurysms (11, 84, 116, 179, 186, 199). TIMP-1 is expressed prominently in aortic aneurysm tissue (11, 116), TIMP-2 is secreted constitutively, and TIMP-3 increases as in atherosclerotic plaques (11). Outward vessel remodeling also correlates with accumulation of foam cell macrophages and increases in MMP-2 and -9 expression after carotid ligation in ApoE knockout mice (122).

Based on extensive histological evidence, macrophage-derived MMPs are particularly implicated in plaque instability. In addition, an increase in the proportion of macrophages in relation to VSMC occurs in areas of plaques prone to rupture and also in aneurysms. Furthermore, isolated macrophages secrete an excess of active MMPs over TIMPs (50). Rabbit foam cell macrophages isolated from atherosclerotic plaques produce MMPs-1 and -3, while alveolar macrophages do not (89). Furthermore, decreases in MMP levels closely parallel reduction of macrophage numbers in rabbit atherosclerotic plaques after cessation of cholesterol feeding or use of cholesterol-lowering drugs (2, 3).

Even though they fall short of direct proof, these correlative studies provide persuasive evidence that MMPs, particularly from macrophages but also from EC, VSMC, and other inflammatory cells, mediate the loss of matrix that underlies weakening of the fibrous cap and provoke plaque rupture.

C. Effects of MMP Overexpression and TIMP Knockout

MMP-1 is not widely expressed in the mouse, where MMP-13 may substitute. This species difference prevents the use of MMP-1 knockout but presents a unique opportunity to investigate the effect of transgenic overexpression of MMP-1. D’Armiento and colleagues (150) targeted MMP-1 overexpression to macrophages, and this had no effect on migration. Somewhat surprisingly, MMP-1 overexpression reduced the progression of atherosclerosis in ApoE knockout mice (150), perhaps because of less collagenous matrix accumulation. MMP-1 overexpression did not cause plaque ruptures, arguably because the plaques were less advanced in the transgenic mice. This experiment illustrates the potential complexities of manipulating a system where MMPs have a dual role in plaque growth by VSMC migration and matrix deposition and instability by matrix destruction. It would be interesting to know the effect of MMP-1 overexpression in already established plaques.

Two groups have examined the effect of TIMP-1 knockout, which was shown directly to increase local vessel wall MMP activity, in the ApoE knockout mouse model (151, 254). Somewhat discordant results, either no change or a 30% decrease, were found in the extent of aortic atherosclerosis. However, both groups found that TIMP-1 knockout significantly increased destruction of medial elastic fibers (Fig. 10), i.e., pseudoaneurysm formation (151, 254). The results support a pathogenic role for MMPs in atherosclerotic aneurysm formation. How-
ever, no luminal plaque ruptures were observed in the aorta in either study (151, 254). In unpublished studies, we also observed no effect of TIMP-1 knockout on ruptures in the brachiocephalic artery. Given the presence of other TIMPs in these knockout models, the results are perhaps to be expected. They do, however, expose an interesting mechanistic difference between aneurysm formation and plaque rupture, at least in the mouse.

D. Effects of MMP Inhibition and Knockout

The effect on atherosclerosis of systemic delivery of an adenovirus that caused expression of human TIMP-1 was investigated in ApoE knockout mice fed a lipid-rich diet. This increased plasma TIMP-1 levels for at least 28 days and reduced aortic plaque area measured 4 wk later in groups of four mice by ~30% (231). Qualitative histological analysis suggested that TIMP-1 overexpression enhanced collagen, elastin, and smooth muscle cell α-actin content and reduced macrophage number, all consistent with greater plaque stability. In contrast, oral administration of synthetic MMP inhibitors showed no effect on plaque development in LDL receptor knockout mice (215) after dietary intervention or angiotensin II treatment (178). Hence, TIMP gene transfer had a greater effect on atherosclerosis than synthetic MMP inhibitors, as found for neointima formation. The contribution of individual MMPs to plaque formation and stability is only beginning to be investigated by knockouts. MMP-3, ApoE double knockout mice had increased collagen and decreased macrophage content in plaques consistent with greater stability, although plaque size was increased overall (255). MMP-9 knockout decreased plaque size as well as macrophage and collagen content in the aortas of chow-fed ApoE knockout mice, which suggests a beneficial effect of this knockout also (169). In the same study, MMP-12 knockout had no effect on plaque size or composition in the aorta (169). In preliminary studies of the brachiocephalic artery, we also observed no effect of TIMP-1 knockout on ruptures in the brachiocephalic artery.
Efficient degradation of collagens, proteoglycan core proteins, and glycoproteins clearly requires the substrate repertoire of several MMPs. In addition, MMPs need to act in concert with each other and with other classes of protease to completely degrade ECM components. An example of this is fibrillar collagen degradation, where MMPs-1, -2, -8, -13, and -14 can mediate only the initial cleavage of triple-helical domains to one-fourth and three-fourths fragments, but other proteases are needed to complete degradation (30). In addition, proteases act together in an activation cascade (Fig. 3). Hence, conditions leading to the coordinated upregulation of several MMPs (Fig. 4), and other proteases, are those most likely to be associated with destruction of the extracellular matrix (51). This principle was clearly demonstrated in the study by Baxter and colleagues (166), where knockout of either MMP-2 or -9 was sufficient to prevent calcium-induced aortic aneurysm formation in mice. MMP-9 from macrophages and MMP-2 most likely from VSMC cooperated to mediate matrix degradation, either directly or by activating other proteases (166).

Atherosclerosis is a complex process, which MMPs could influence in a host of ways. Gelatinases presumably facilitate VSMC phenotypic modulation and migration in the same way in atherosclerotic plaques as they do consistently in other models of intimal growth. From studies in knockout mice, MMP-12 appears to have an important role in invasion of macrophages into ECM (250). The phenotype of MMP-9 knockout mice also includes a deficit in bone growth-plate invasion by monocytes and capillaries (284). Indeed, MMPs have an established role in angiogenesis (212), which appears to contribute to plaque growth (193) as well as being a histological feature associated with unstable plaques (127, 185). Hence, MMPs could influence plaque stability indirectly through any of these processes in addition to direct effects on degradation of ECM components.

Non-ECM substrates might also be involved. For example, the ability of MMP-11-mediated degradation of α₂-antiplasmin and α₁-antitrypsin could increase activity of cathepsins, plasmin, and leukocyte elastase (241).

F. Conclusions

In principle, several MMPs (and other proteases) with complementary substrate repertoires should be needed to mediate plaque rupture. Descriptive and correlating studies consistently demonstrate MMP overexpression and matrix destruction at the macrophage-rich regions of atherosclerotic plaques that are prone to rupture. On the other hand, early fatty streaks also show upregulation of MMPs, which could therefore be involved in plaque building by intimal expansion as well as plaque rupture by matrix destruction. TIMPs are upregulated, especially at the base of plaques, and in part counteract the destructive potential of MMPs. Gene disruption and
overexpression studies in atherosclerosis-prone mice suggest essential roles for MMPs-2, -3, -9, and -12 in atherosclerosis formation, but only preliminary data are yet available with regard to plaque rupture. Studies in progress, for example, in the ApoE knockout mouse brachiocephalic artery model, should further clarify the role of individual MMPs.

IX. PHARMACOTHERAPY

The possible routes for reducing MMP activity during intimal thickening and in atherosclerotic plaques comprise 1) direct inhibition of enzyme activity and 2) inhibition of MMP production.

A. Direct Inhibition by Synthetic Inhibitors and Gene Therapy with TIMPs

There is already some direct clinical experience with MMP inhibitors. MMP inhibitors have been given for quite prolonged periods to patients with advanced malignancies, albeit with no proven benefit (61). Early trials revealed the somewhat unexpected side effect of tendonitis, although this was avoided by dose adjustment and different drug specificity in other studies. Doxycycline, an antibiotic and broad-spectrum MMP inhibitor, is quite widely used to treat acne and in malarial prophylaxis; it has been given orally to patients with asymptomatic aortic aneurysms for up to 6 mo (214). The action of broad-spectrum MMP inhibitors to prevent intimal expansion could therefore be evaluated in clinical trials.

However, almost all preclinical trials with broad-spectrum MMP inhibitors failed to show long-term reduction of neointima formation after balloon injury with or without stenting (24, 54, 215, 265, 312). In most cases, other classes of protease appear to substitute the function of MMPs, particularly in proliferating VSMC (1), and there is therefore catch-up after the effect of early MMP inhibition (24). Only Li et al. (154) showed a significant reduction in in-stent restenosis at 10 wk in a rabbit double-injury model. An open label trial of an MMP inhibitor-eluting stent (The Brilliant 1 study) was halted early because it did not apparently show benefit. On the other hand, MMP inhibitors decrease constrictive remodeling after angioplasty without stenting in pigs (68) so that a human trial in this context could still be justified.

The relatively primitive state of development, high relative production costs, and complex regulatory issues surrounding TIMP gene therapy are obstacles to its clinical application. However, proof of principle studies are still valuable because slowly developing pathologies, in particularly vein-graft disease, might be more amenable to gene therapy than one-off local drug treatments. In addition, veins used for grafting can be treated ex vivo before reimplantation, and hence, exposure of the patient to therapeutic reagents can be minimized.

Given the dual role of MMPs in fibrous cap formation (i.e., intimal growth) and cap rupture (i.e., matrix destruction), the effect of a broad-spectrum MMP inhibitor on acute coronary events is difficult to predict. Nevertheless, further preclinical studies of MMP inhibitors and knockouts in models of unstable atherosclerosis are clearly appropriate. Greater knowledge of the specific MMPs or groups of MMPs causing plaque instability will justify trials of inhibitors with the correct specificity.

B. Inhibition of MMP Production

Quercitin, a dietary flavonoid related to those present in red wine, inhibits MMP-9 secretion by effects on AP-1 and NF-κB transcription factors (190). Several other physiological mediators probably suppress MMP induction locally in the vasculature. Heparin and HSPG inhibit the release of several proteases, including MMPs from VSMC (13, 135). This implies that the basement membrane could suppress MMP secretion from normal EC and VSMC. Reports regarding the effects of nitric oxide are conflicting. Nitric oxide was reported to tonically inhibit MMP-9 but not MMP-2 production from VSMC in one study (278) but to enhance stimulated MMP-9 expression in another (180). A recent study shows that nitric oxide inhibits MMP-2 secretion in EC by activating the repressor protein ATF3 (52). TGF-β inhibits induction of MMP-1, -3, and -7, although paradoxically upregulates MMP-13, at least in fibroblasts (279). TGF-β also inhibits MMP-9 secretion in mast cells (78) and MMP-7 (39) and MMP-12 (80) secretion in macrophages. MMP-7 secretion is also inhibited by IL-4 and IL-10 (39). Given the broad range of MMPs induced by the CD40 ligation in isolated cell cultures (173, 176, 241–243, 300), one may speculate that interruption of this signaling system would have a profound effect in MMP activity in plaques. Physiologically, interferon-α and/or interferon-γ inhibit the CD40 ligation-induced secretion of MMP-1 and -9 from VSMC (243) and MMP-1, -3, -9, and -12 from macrophages (170, 300).

MMPs-1, -3, -7, and -9 are all downregulated by nuclear hormone receptor (NHR) agonists, which include corticosteroids, retinoids, thyroid hormones, and sex hormones (238). Steroid hormones also stimulate TGF-β1 production (50), which indirectly inhibits MMP induction, as described above. Activation of the Liver X receptor (LXR) inhibited MMP-9 but not MMP-12 or -13 secretion from mouse bone marrow-derived macrophages stimulated with lipopolysaccharide, TNF-α, or IL-1β (48). The mechanism was apparently not by direct binding to the MMP-9 promoter, and while it was downstream of NF-κB activation, LXR activation did not prevent activation or binding of NF-κB (48). Perhaps of greatest current clinical
relevance are peroxisome proliferator-activated receptor (PPARs), transcription factors that also belong to the NHR superfamily, that are widely expressed in vascular cells (182, 183, 226). PPARs and -γ ligands selectively inhibit phorbol ester-induced MMP-9 secretion by VSMC (183), monocytic cell lines (253), and monocyte-derived macrophages (182). Moreover, treatment of diabetic patients with the PPARγ ligand rosiglitazone reduced plasma MMP-9 levels within 2 wk, implying direct inhibition of MMP-9 secretion at a pharmacologically relevant concentration in vivo (181). Inhibition of MMP-9 production might therefore be an important component of the pleiotropic effects of PPAR ligands, which are already in trial for prevention of acute coronary events (18).

Statins inhibit mevanolate formation by hydroxymethylglutaryl (HMG)-CoA reductase, the rate-limiting step in cholesterol and isoprenoid synthesis. Their cholesterol-lowering effects are associated with reduction of unstable coronary events in several large clinical trials (219). Statins dramatically inhibit secretion of MMP-1, -3, and -9 from macrophages and MMPs-1, -2, -3, and -9 but not TIMP-1 or -2 from VSMC (3, 20, 168). The mechanism is posttranscriptional, overcome by mevanolate, and mediated by inhibition of prenylation (168). The effects of cerivastatin are obtained at 10–50 nM, within the range of concentrations obtained in humans (143) and known to cause other non-lipid-lowering effects (reviewed in Refs. 208, 269). Statins reduce MMP protein expression and activities when administered to hyperlipidemic rabbits, and this changes plaque morphology consistent with increased stability (2–4). Statins also decrease markers of instability in the coronary arteries of cynomolgus monkeys (297) and in the brachiocephalic artery of mice (19) independently of lipid-lowering effects. Hence, a direct effect on MMP secretion could well contribute.

X. SUMMARY: WHAT MEDIATES THE TRANSITION FROM PHYSIOLOGICAL REMODELING TO MATRIX DESTRUCTION?

From the work cited above, MMPs have been implicated both in intimal thickening, which involves net matrix deposition, and plaque rupture, which involves net matrix destruction. Indeed, there is substantial supporting evidence for both propositions. If both are correct, the idea merits consideration that MMP activity is dysregulated during the transition from plaque building to plaque disruption.

The null hypothesis is that there is no dysregulation as such, merely that MMP activity is appropriately regulated but occurs in the wrong place at the wrong time relative to chance mechanical factors. Early matrix destruction is a prerequisite for intimal expansion because it permits entry of inflammatory cells and frees VSMC to migrate, change phenotype, and proliferate. Later, there is fibrosis that more than compensates for the initial destruction, the same sequence observed for example in dermal wound healing. Consistent with this, fibrous cap formation is a late event in atherosclerosis. If the shoulder regions of plaques simply represent new, active areas of plaque growth, the status of their matrix might simply reflect the early (destructive) stages of remodeling. Were it not for a mechanical accident leading to rupture, these areas would also eventually become fibrous and safe. The corollary of this null hypothesis is that inhibiting plaque progression will inevitably stabilize plaques, a concept that fits well with the results from lipid-lowering trials (219).

A second hypothesis is that matrix destruction reflects an imbalance between the production of “good” and “bad” MMPs. Gelatinases (MMP-2 and -9) could represent good MMPs because basement membrane degradation plays a key role in liberating contractile VSMC to participate in repair. The effect of MMP-9 knockout to decrease VSMC migration and repair after vascular injury (56, 86) and increase plaque rupture (129) is consistent with this idea. On the other hand, collagenases (e.g., MMPs-1, -8, and -13) and stromelysins (e.g., MMPs-3 and -7) are generally thought of as bad MMPs because they are closely associated histologically with degraded collagen in areas prone to plaque rupture (268). The corollary of this hypothesis is that selective inhibitors of some MMPs might be an effective treatment for plaque instability that would spare repair mechanisms. Conversely, broad-spectrum MMP inhibitors might be ineffective, as suggested by the current experimental evidence (215), or even harmful. These ideas could be investigated by a comprehensive survey of drug treatments and MMP knockouts in the same standardized model of plaque growth and rupture. Even so, the results could only be cautiously extrapolated to humans.

A third hypothesis is that a greater extent of expression of the same MMPs present during physiological remodeling can lead to aneurysms or rupture. Data that MMP-9 knockout reduces neointima formation after mouse carotid artery injury (56, 86) but also protects against aneurysm formation in other mouse models (166, 217) fit with this hypothesis. It could be tested directly by selectively overexpressing individual MMPs in a suitable animal model. The corollary of this hypothesis is that MMP inhibitors should be an effective treatment. Second, it focuses attention on the factors that might be driving MMP overexpression, which are most commonly believed to be inflammatory and immune phenomena.

A fourth hypothesis that we recently proposed (51) is that increasingly diverse stimuli (i.e., injury, inflammation, immune mechanisms, oxidative stress) upregulate an ever-broader spectrum of MMPs. This eventually includes members of each of the gelatinase, interstitial...
collagenase,stromelysin,metalloelastase,and MT-MMP subfamilies. Because of the limited capacity of individual MMPs to remodel the ECM, a broad spectrum of MMPs (and indeed other proteases) would be more likely to catalyze matrix destruction rather than physiological remodeling. Given the overlapping specificities of the individual MMPs, there is likely to be a high degree of redundancy. The implication of this hypothesis is that targeting individual MMPs might have a relatively minor impact on pathology, whereas targeting the processes that upregulate several MMPs (e.g., inflammatory mediators, oxidative stress) might be more effective.

Whether on not MMPs provide a new route to therapies, just over 10 years of intensive MMP research has contributed to a revolutionary change in the way we think about blood vessels. This new perspective counters the notion that the ECM is simply an amorphous filler between the cells, which responded on demand to growth factors. It emphasizes that the behavior of all vascular cells, indeed their very composition, is dictated by connections principally with the ECM and with each other through cell contacts. Binding to the ECM can block growth factor availability, and signals from the ECM and cell contacts can completely negate the effect of growth factors. In turn, the rate-limiting process of proteolysis, in which MMPs are key players, regulates the composition of ECM as well as many cell-to-cell contacts. Hence, according to this view, MMPs and other extracellular proteases are as important as growth factors in their ability to orchestrate the behavior of vascular cells. The very importance of extracellular proteolysis is probably responsible for the complexity of the MMP system and its inherent redundancy. Much as the internet is robust because of its many nodes, extracellular proteolysis is reliably effective because of the concerted action of many overlapping components. As such, it will be a challenge to identify specific roles for each of the MMPs. Instead, ingenious experimentation may be needed to unravel the web of interactions between individual MMPs and between MMPs and other proteases. Nevertheless, this research will be driven forward by the importance of its goals and the many opportunities for new insights.

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