

Proteolysis of the Pericellular Matrix

A Novel Element Determining Cell Survival and Death in the Pathogenesis of Plaque Erosion and Rupture

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Abstract—The 2 major general concepts about the cell biology of atherogenesis, growth of smooth muscle cells, and lipid accumulation in macrophages, ie, foam cell formation, have not been able to satisfactorily explain the genesis of acute coronary syndromes. Rather, the basic pathology behind the acute atherothrombotic events relates to erosion and rupture of unstable coronary plaques. At the cellular level, we now understand that a switch from cellular growth to cellular death, notably apoptosis, could be involved in turning at least some types of atherosclerotic plaques unstable. Because intimal cells require a proper matrix environment for normal function and survival, the vulnerability of an atherosclerotic plaque may critically depend on the integrity of the pericellular matrix of the plaque cells. In vitro studies have revealed that plaque-infiltrating inflammatory cells, such as macrophages, T-lymphocytes, and mast cells, by secreting a variety of proteases capable of degrading pericellular matrix components, induce death of endothelial cells and smooth muscle cells, and so provide a mechanistic explanation for inflammation-dependent plaque erosion and rupture. Thus, a novel link between inflammation and acute coronary syndromes is emerging. For a more explicit understanding of the role of proteases released by inflammatory cells in the conversion of a clinically silent plaque into a dangerous and potentially killing plaque, animal models of plaque erosion and rupture need to be established. (*Arterioscler Thromb Vasc Biol.* 2004;24:1350-1358.)

Key Words: proteases ■ pericellular matrix ■ apoptosis ■ plaque erosion ■ plaque rupture

In the present review, we focus on the proteolytic mechanisms in the arterial intima regulating matrix remodeling and cellular death in the detrimental processes, which makes a plaque vulnerable to erosion or rupture (Figure 1). In contrast to previous recent reviews, which have discussed the role of extracellular matrix (ECM) remodeling in plaque rupture,^{1,2} the present review discusses the role of the pericellular matrix (PCM), a major regulator of cell survival and death, in plaque erosion or rupture. However, because most of the present results concerning the elucidation of the mechanisms of proteolysis and plaque erosion or rupture have been obtained under in vitro conditions, one has to be careful when extrapolating the results to the processes actually happening in vivo.

Formation of Atherosclerotic Plaques as a Response to Injury

The original “response to injury” hypothesis by Rudolf Virchow (dating back to 1856) was revived during the period extending from 1960s until the end of the century by the giant of modern cell biology of atherogenesis, the late Dr Russell Ross. According to this hypothesis, phenotypic conversion of smooth muscle cells (SMCs) into “synthesizing SMCs” with an increasing capacity to divide and synthesize ECM is the

culminating event in the development and advancement of an atherosclerotic plaque.^{3,4} A necessary parallel to this axiom is that a slow division of SMCs (division also referred to as “growth” of SMCs in the text) and slow secretion of ECM components by the SMCs are beneficial processes. These important cellular studies, originating in the discovery of a platelet-derived growth factor (PDGF), found their animal counterparts in studies with primates and swine, which were conducted under conditions of artificially stimulated SMC growth, ie, after mechanical denudation (erosion) of the endothelium.⁴ Thus, endothelial injury followed by platelet adhesion, aggregation, and ensuing secretion of PDGF as a “response to injury” triggered SMC proliferation and the formation of a secondary restenotic lesion. The clinical validity of the concept of restenosis as a “response to injury” has been confirmed in humans after invasive treatment of primary atherosclerotic lesions, ie, when the primary lesions are treated by angioplasty and secondary restenotic lesions are formed. The final proof for a critical role of SMC division in this process has been obtained very recently by local delivery of antiproliferative agents, which effectively prevent the development of restenosis.⁵

In sharp contrast to such secondary restenotic lesions, we do not have any safe and powerful pharmaceutical tools

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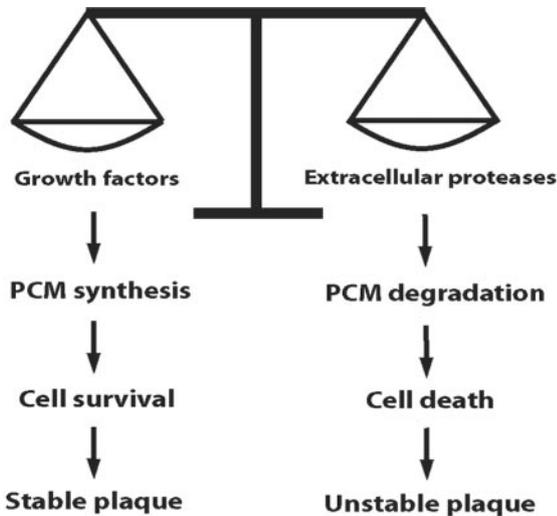


Figure 1. Schematic picture depicting the effect of intrinsic factors on plaque stability via regulation of the integrity of pericellular matrix (PCM) of ECs and SMCs of the fibrous cap. The intrinsic factors (growth factors and extracellular proteases) determine the balance between cell survival and death in an atherosclerotic plaque. By degrading PCM components necessary for cell survival, the proteases secreted or activated by infiltrating inflammatory cells render an atherosclerotic plaque vulnerable to erosion or rupture.

available that would prevent SMC proliferation in the coronary arteries when the primary or spontaneous atherosclerotic lesions emerge. Nor do we know when and how such drugs should be administered to human beings, if at all. In terms of primary atherosclerosis, the SMC growth hypothesis primarily applies to human fibrotic plaques, which, in contrast to restenosis after angioplasty, are usually slowly growing and may contribute to stable narrowing of the coronary lumen that ultimately manifests itself as exertional angina. Indeed, in the spontaneous atherosclerotic lesions of human coronary arteries, the rate of SMC division is extremely low.^{6–8} Moreover, SMC growth and survival in the lesions (at least in the cap region) is considered to stabilize plaques and so should prevent plaques from rupturing.⁹ Based on these considerations, the lack of interest in planning new strategies for the prevention of spontaneous atherosclerosis by blocking SMC growth is reasonable and easy to understand.

The Lipid Hypothesis of Atherogenesis

The other major hypothesis of atherogenesis, the lipid hypothesis, was born in the early years of this past century (1914) when Anitschkow fed cholesterol to rabbits and found that high plasma cholesterol levels caused cholesterol to accumulate also in the arterial wall. The lipid hypothesis of atherogenesis has dominated the field of atherosclerosis research ever since, and the elegant experiments with cultured cells leading to the discovery of the mechanism of formation of foam cells have provided strong new impetus to the lipid hypothesis.^{10,11} The powerful drugs of today with their significant dyslipidemia-correcting effects and ensuing clinical benefits urge us to believe that the lipid hypothesis is going to survive and even dominate the field of atherosclerosis research in the future. The additional anti-inflammatory

pleiotropic effects of the lipid-lowering drugs point to the important role of inflammation, also related to local lipid accumulation, as a pathogenic principle of atherogenesis.

However, the 2 described general concepts about the cell biology of atherogenesis, SMC growth and foam cell formation, have not been able to satisfactorily explain the molecular pathogenesis of acute coronary syndromes (ACSs). The basic pathology behind the majority of the cases of ACSs relates to unstable coronary plaques, which trigger the acute atherothrombotic events. The majority of the ACSs that occur today are caused by erosion or rupture of a vulnerable coronary plaque. Plaque erosion describes a local superficial injury of the plaque and primarily affects the endothelial cells (ECs) and their pericellular matrix. Plaque rupture, again, primarily affects SMCs, and secondarily affects ECs, and describes a deeper injury in the plaque. Plaque rupture accounts for $\approx 70\%$ of the fatal acute myocardial infarctions and sudden coronary deaths, whereas plaque erosion accounts for $\approx 30\%$.¹² The risk of plaque erosion or rupture appears to be a function of both intrinsic conditions within the plaque, which determine plaque vulnerability, and extrinsic conditions, which may actually trigger plaque erosion or rupture.

The intrinsic plaque weakening conditions are largely determined by the size of the lipid-rich necrotic core, the thickness of the fibrous cap separating the core from the circulating blood, and the presence of ongoing inflammation within the cap.¹² The actual intrinsic mechanisms that predispose atherosclerotic plaques to erosion or rupture include local degradation of ECM components and increased proapoptotic signals, both in the subendothelial space and in the deeper regions of the fibrous cap, followed by dysfunction and death of ECs and SMCs. The timing and location of plaque erosion or rupture is then determined by extrinsic conditions, such as local stress and hemodynamic forces, including cyclic stretching, compression, bending, flexion, shear, and pressure fluctuations.¹³ Turbulent flow and low fluid shear stress may also directly affect the gene expression profile in endothelial cells and predispose to the activation of proatherosclerotic and proinflammatory genes, such as NF- κ B and c-Jun N-terminal kinase.^{14,15} Lack of such strong local forces may be one reason why advanced vulnerable plaques, as a rule, fail to erode or rupture in animal models of atherosclerosis.¹⁶ However, despite differences in the actual mechanisms by which thrombus formation is triggered as a consequence of erosion or rupture, the presence of a vulnerable plaque is essential in the ultimate atherothrombotic process leading to ACSs and sudden cardiac death.¹²

What Makes an Atherosclerotic Plaque Vulnerable to Erosion and Rupture?

For a plaque to become vulnerable, intrinsic modifications in the 3 components of a plaque, the fatty component, the SMC-ECM component, and the endothelium, have to take place. First, death of the lipid-filled foam cells will initiate the growth of an extracellular necrotic lipid core,¹⁷ which is then separated by a fibrous cap from the arterial lumen. An absolute prerequisite for the formation of a fibrous cap is the preceding formation of a necrotic lipid core: a cap without a core does not exist. Second, death of the SMCs in the formed

fibrous cap will reduce their number and so impair the production of ECM in the cap, rendering it thin and vulnerable to rupture. Third, endothelial dysfunction and death will predispose to erosion and an immediate onset of atherothrombotic events. Taken together, at the cellular level, we now understand that cellular growth (SMCs) makes a plaque stable against rupture, whereas cellular death (foam cells, SMCs, and ECs) makes a plaque potentially unstable. Hence, a switch from cellular growth to cellular death caused by intrinsic modifications appears to be in a key position in turning an atherosclerotic plaque vulnerable, ie, prone to erosion or rupture.¹⁸

This dichotomized view about the relationship between the genesis of vulnerable plaques, ie, SMC death leading to vulnerable and SMC growth leading to stable plaques, primarily applies to lesions without significant flow obstruction. These include early and intermediate lesions and advanced lesions growing “outwards,” all of which are associated with normal or only slightly narrowed luminal diameter, therefore allowing more or less laminar flow to sustain.⁸ In contrast, lesions that are highly stenotic and cause significant narrowing of the lumen are exposed to potentially detrimental extrinsic factors, such as turbulent blood flow, and may undergo sudden waves of rapid growth as a response to recurrent erosion caused by the aberrant hemodynamic forces. Here, mechanical erosion with ensuing exposure of the subendothelial thrombogenic surface leads to PDGF release from attached platelets and acutely induced SMC division, like after mechanical denudation of the endothelium (angioplasty). Thus, in a subset of highly advanced plaques, numerous proliferating SMCs and thickening of the cap is not associated with plaque stability, but rather with recurrent transient instability of the plaque.¹⁹

The Role of Apoptosis in Plaque Erosion and Rupture

As outlined, programmed cell death or apoptosis is emerging as a novel intrinsic determinant in the development of vulnerable plaques prone to erosion or rupture.^{7,20–22} In addition to apoptosis of foam cells, of SMCs, and of ECs, apoptotic death of infiltrating inflammatory cells eg, monocyte-derived macrophages, T-lymphocytes, and mast cells, may be of importance in determining the stability of the atherosclerotic plaque. Despite differences in origin and function, the cell types present in the atherosclerotic intima may share final common pathways and mechanisms of cell survival and apoptosis, which are governed by cell type-specific pro-apoptotic and antiapoptotic components present locally in the atherosclerotic intima.

Several potential mechanisms, by which apoptosis may occur in atherosclerotic plaques, have been described. The lipid component itself, ie, low-density lipoprotein (LDL), when modified in the intima is capable of inducing apoptosis of the intimal cells. Oxidized LDL (ox-LDL) and oxysterols have proven capable of inducing apoptosis in SMCs.^{23,24} Also, the presence of cytokines, notably the death ligands (FasL and tumor necrosis factor (TNF)- α), and the loss of growth factors (insulin-like growth factor-1, transforming growth factor [TGF]- β) have been shown to induce apoptosis

in plaque-derived SMCs.²⁵ Furthermore, angiotensin II, the effector peptide of the renin-angiotensin system, and reactive oxygen species are capable of triggering apoptosis in ECs.²⁶ A new concept of cell death in the arterial wall is that local pathological remodeling of the ECM by proteases may induce apoptosis of the medial and intimal cells.² This subset of apoptosis, in which cell death is induced by the loss of cell–matrix interactions, has been called anoikis and is thought to play a role in physiological processes regulating tissue and cell homeostasis, as well as in developmental and oncogenic processes.²⁷

The Matrix as a Regulator of Apoptosis and Plaque Stability

The intimal cells are adherent cells and thus require a proper ECM environment for normal function and survival.^{27–30} In addition to being embedded in an ECM network, most adherent cells are surrounded by a pericellular matrix (PCM) that consists of ECM molecules closely associated with the plasma membrane of the cell. Accordingly, PCM is the region of contact between the cell surface and the more distant ECM. Because PCM is also a major site of ECM assembly and remodeling, it is evident that both the quality and the quantity of the PCM is important for cell viability in the plaque and also is a major determinant of plaque stability. Both the PCM and the ECM in the atherosclerotic plaque are produced locally by the intimal cells. The ECM consists of a solid network of structural molecules, notably fibrillar collagen, elastin, and proteoglycans, which give the arterial wall its tensile strength and elasticity.³¹ The PCM consists of adhesive glycoproteins, such as fibronectin, vitronectin, and laminin, known for their ability to promote cell adhesion and cell survival.³¹ Thus, in terms of cell survival and death, the metabolism of the closely connected PCM likely governs the fate of an individual cell more than the distant ECM. At the cellular level, the PCM-mediated cell survival mechanisms are controlled and modulated by integrins, which are heterodimeric integral cell surface receptor proteins embedded in the plasma membrane of adherent cells.^{32,33} By forming different heterodimers consisting of combinations of various α_x - and β_x -integrin subunits, the integrins form focal contacts, ie, membrane assembly sites for cytoskeletal and cell signaling components, in which they recognize and interact with specific arginine-glycine-aspartate sequences present in many target glycoproteins of the PCM.^{32,33}

Because the integrin-mediated PCM/cell interaction directly influences the intracellular signaling cascades, ie, the “outside-in signaling,” the integrins act as cellular biosensors that regulate biological processes, such as cell growth, differentiation, migration, and survival.³⁴ Thus, integrin-mediated activation of the nonreceptor tyrosine focal adhesion kinase (FAK) and downstream signaling events is crucial for cell growth and survival.³⁵ The downstream pathways include phosphoinositide 3-kinase (PI3K), protein kinase B (PKB/Akt), and mitogen-activated protein kinase (MAPK) pathways, such as the extracellular signal regulated kinase (ERK)1/2. FAK has also been shown to regulate the expression of antiapoptotic members of the bcl-2 family, which preserve the mitochondrial integrity, and FAK also directly

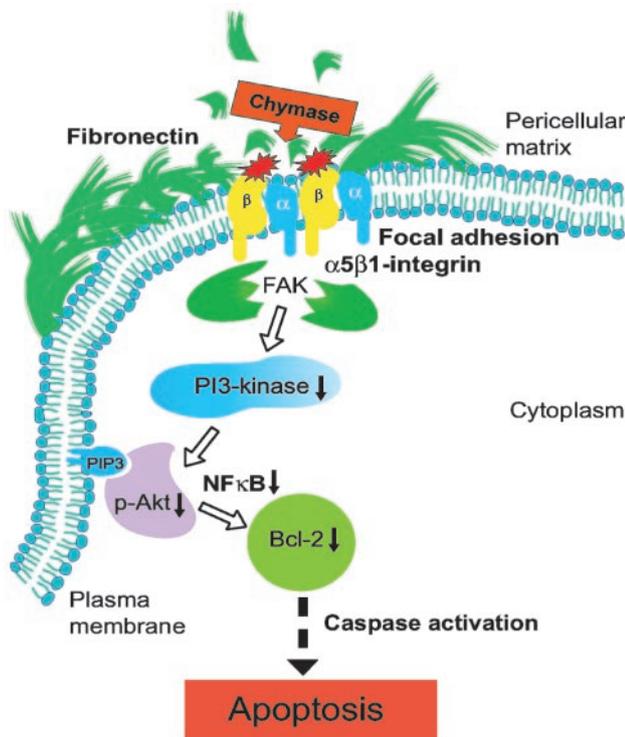


Figure 2. Schematic model illustrating the proposed mechanism of chymase-induced apoptosis in SMCs. Chymase degrades the fibronectin component of the pericellular matrix, disturbing the fibronectin- $\alpha_5\beta_1$ integrin interaction, with subsequent degradation of FAK and loss of focal adhesions. As a consequence, the Akt signaling cascade is dephosphorylated and NF κ B is degraded, leading to downregulation of antiapoptotic bcl-2 and activation of caspases.

regulates caspase inhibitors, notably members of the inhibitor of apoptosis (IAP) family, via PI3K/Akt-mediated activation of the NF- κ B pathway.^{36,37} Because caspases are the executioner proteases responsible for many of the death-directed events in the apoptotic process, disruption of the caspase-inhibiting FAK signaling pathways rapidly induces apoptosis. The role of integrin-matrix interactions in cell survival is further emphasized by the fact that integrins also associate with growth factor receptors and act as important cofactors in growth factor-mediated stimulation of intracellular survival-signaling pathways.^{38,39} Thus, cell survival critically depends on the interaction between integrins and PCM and, as exemplified in our *in vitro* model of mast cell protease-induced apoptosis of human coronary artery SMCs and ECs, loss of integrity of the PCM will disturb the integrin-dependent survival signaling pathways, leading to caspase activation and, ultimately, to apoptosis (Figure 2).

In both normal and atherosclerotic coronary arteries, matrix turnover is regulated at the level of synthesis and degradation.⁴⁰ The synthetic activity is controlled by locally secreted and activated growth factors, such as TGF- β and basic fibroblast growth factor (bFGF), as well as by peptides of the neurohormonal system, including angiotensin II. In contrast, degradation of the matrix occurs through the action of different enzymatic mechanisms, notably through elastolytic and proteolytic enzymes, secreted locally by the intimal cells.⁴¹ Matrix metalloproteinases (MMPs) and cysteine pro-

teases, such as cathepsins, are the major elastolytic enzymes capable of degrading the structural components of the ECM. In addition, neutrophil elastase⁴² and mast cell chymase⁴³ and tryptase⁴⁴ are neutral serine proteases capable of efficiently degrading adhesive protein components of both the PCM and ECM. In the normal intima, synthesis and degradation of PCM and ECM are in balance, whereas in the atherosclerotic intima, and especially in vulnerable plaques, matrix degradation dominates.⁴⁵ Hence, extensive degradation of the structural components of the ECM in the plaque, with loss of tensile strength and elasticity, as well as degradation of the PCM with disruption of outside-in-mediated cell survival signaling, are potential mechanisms for the weakening and rupture of the atherosclerotic plaque.

Which cells are involved in the degradation of PCM and ECM in the atherosclerotic plaque? By secreting MMPs and activating urokinase plasminogen activator and plasmin, the synthesizing SMCs may themselves contribute to PCM/ECM degradation.⁴⁶ Although plasmin secreted by SMCs may under certain conditions lead to their apoptotic death,⁴⁷ the secretion of proteases by plaque SMCs usually reflects a physiological response necessary for focalized pericellular proteolysis required for their division and movement, similar to that described for the migration of other cell types.⁴⁸ Therefore, it is unlikely that SMCs are the major PCM and ECM degrading cells in the plaque, especially because their number is significantly reduced in vulnerable atherosclerotic plaques. A more likely explanation is that the infiltrating inflammatory cells synthesize and secrete the proteolytic enzymes responsible for the detrimental degradation of PCM and ECM in the vulnerable areas of the plaques.

The Role of Macrophages in Plaque Erosion and Rupture

Macrophages represent the most powerful inflammatory component in atherosclerosis and are present in early and advanced lesions, where they scavenge modified LDL particles and accumulate intracellular cholesterol. However, as a response to modified LDLs, such as ox-LDL, the macrophages synthesize and secrete MMPs, notably MMP-1 and MMP-9.^{49,50} MMPs, by being able to degrade all types of ECM components, are thought to play a prominent role in atherosclerosis and to contribute to cap rupture and erosion.^{51,52} In addition, by secreting cytotoxic compounds, such as TNF- α , FasL, and angiotensin II, infiltrating macrophages may further activate the secretion of MMPs through autocrine and/or paracrine mechanisms on neighboring cells.⁵³ The infiltrating macrophages may also directly reduce ECM synthesis in the atherosclerotic plaque by inducing FasL-mediated SMC apoptosis.⁵⁴

Although macrophages are specialized phagocytes, ie, capable of removing modified LDLs and apoptotic cell debris by scavenger receptor-mediated phagocytosis, an excess of ingested toxic substances may eventually lead to macrophage dysfunction, followed by their apoptotic or necrotic death.⁵⁵ Cholesterol in excess, with subsequent trafficking to the endoplasmic reticulum membranes and activation of the C/EBP homologous protein-10 arm of the unfolded protein response, has been proposed as the key signaling step in

cholesterol-induced apoptosis in macrophages.⁵⁶ Moreover, feeding macrophages in vitro with ox-LDL and associated oxysterols resulted in a destabilization of the lysosomes with leakage of cathepsins,⁵⁷ which may, again, activate caspase-3 and cause extensive apoptosis of the macrophages.⁵⁸ Such ox-LDL-mediated apoptosis has been shown to involve the surface receptor LOX-1, which is extensively expressed in advanced atherosclerotic carotid arteries and colocalizes with apoptotic cells.⁵⁹ Although survival factors, such as uncoupling protein 2 (UCP2) and caspase-2, are upregulated in response to DNA damage in macrophage-derived foam cells,^{60,61} the balance between macrophage survival and death in atherosclerotic lesions is clearly disturbed in favor of death.^{18,20} Increased apoptosis and reduced phagocytotic capacity may then lead to secondary necrosis, followed by uncontrolled release of proteases capable of further inducing ECM degradation and cell death, ie, a *circulus vitiosus*, contributing to cap weakening and the growth of the necrotic core of an atherosclerotic plaque.⁶²

Expression of elastolytic enzymes, such as cathepsins S, K, and F, is highly increased in atherosclerotic lesions, particularly in areas rich in macrophages^{63,64} and SMCs.⁶² Moreover, cystatin C, the natural inhibitor of such cysteine proteases, is severely reduced in both atherosclerotic and aneurysmal aortic lesions.⁶⁵ These results suggest that lysosomal cathepsins, whether released by macrophages through active secretion or through passive leakage from lysosomes caused by toxic stress and cellular dysfunction, may participate in ECM degradation and plaque rupture.⁶³

Lymphocytes in Plaque Erosion and Rupture

Cytotoxic T-lymphocytes and natural killer (NK) cells may also contribute to ECM remodeling by producing and secreting serine proteases called granzymes, which are capable of degrading ECM components such as fibrillar collagen, laminin, fibronectin, and proteoglycans.⁶⁶ T-lymphocytes may also induce MMP expression in SMCs by a CD40-mediated mechanism further inducing ECM degradation.⁶⁷ Although NK cells are relatively rare in lesions, they have been shown to secrete perforin, a protein capable of introducing the granzymes directly into the cytoplasm of the structural target cells by permeabilizing their plasma membrane.⁶⁸ The granzymes have been shown to activate caspases inside target cells and to trigger their apoptosis. Recently, it was shown that perforin-expressing CD4⁺ T-lymphocytes were significantly increased in peripheral blood of patients with unstable angina and that such cytotoxic CD4⁺ T-lymphocytes effectively killed human umbilical vein endothelial cells in culture.⁶⁹ T-lymphocytes in human atherosclerotic plaques are the major source of interferon- γ ,⁷⁰ and this proinflammatory cytokine is capable of inducing apoptosis in macrophages by upregulating the TNF- α receptor 1 and caspase-8.⁷¹ Importantly, patients with unstable angina have been found to have a higher percentage of both CD4⁺ and CD8⁺ T-lymphocytes expressing CD40L compared with patients with stable angina pectoris or with healthy controls.⁷² Moreover, CD8⁺ T-lymphocytes are found at varying proportions in human atherosclerotic lesions, and they may cause some of the widespread apoptosis associated with atherosclerosis.^{70,73} Fi-

nally, T-lymphocytes in plaques may not only induce apoptosis of other cells but also undergo apoptosis themselves when ox-LDL in the plaques increases their production of FasL.⁷⁴

Mast Cells in Plaque Erosion and Rupture

Mast cells are inflammatory cells best known for their pivotal role in allergic diseases.⁷⁵ They act as "sentinel cells" at the interface of the host and environment and, when activated, trigger acute allergic and ensuing sustained inflammatory reactions.⁷⁶ Mast cells are also present in the arterial wall, where they form part of the inflammatory cell infiltrate and so may contribute to the progression of atherosclerotic lesions.⁷⁷⁻⁷⁹ Increasing evidence shows that mast cells also may play a role in the weakening and rupture of atherosclerotic plaques.⁸⁰

Activated mast cells release an array of mediators, such as neutral serine proteases, cytokines, and proteoglycans,^{76,81} and many of which have pro-inflammatory properties. Mast cell-derived mediators, such as tryptase,⁸² TNF- α ,⁸³ and histamine,⁸⁴ can activate ECs to express adhesion molecules, notably P-selectin and vascular cell adhesion molecule-1, responsible for the recruitment of other inflammatory cells. By secreting TNF- α and TGF- β , mast cells are also able to stimulate the production of MCP-1,⁸⁵ and thus have the potential to induce the infiltration of monocytes into an atherosclerotic plaque. Taken together, these findings raise the possibility that one mechanism by which mast cells may participate in the weakening of the atherosclerotic plaque is recruitment of inflammatory cells into the area.

Mast cells have also been proposed to play a direct role in the weakening and rupture of plaques by increasing MMP synthesis and activation, inhibiting SMC proliferation, and inducing SMC and EC apoptosis.⁸⁰ By releasing TNF- α ,⁸⁶ mast cells can induce the synthesis and release of MMP-9 in an autocrine fashion and in a paracrine fashion from macrophages.^{87,88} Mast cells also synthesize and release MMP-1,⁸⁹ which has been found in atherosclerotic lesions.⁹⁰ Chymase and tryptase, the 2 major neutral proteases of human mast cells, are capable of activating pro-MMP-1⁹¹ and pro-MMP-3,⁹² respectively. In addition, chymase and tryptase can directly degrade PCM components, such as fibronectin and vitronectin.^{43,44} Chymase may also reduce the synthesis of ECM components by inhibiting the expression of collagen in SMCs through TGF- β -dependent or independent mechanisms.⁹³ Furthermore, mast cell-derived heparin proteoglycans have been shown to inhibit the proliferation of rat SMCs in vitro,⁹⁴ suggesting that mast cells could participate in the regulation of SMC growth in the vulnerable plaque.

Several mast cell-derived mediators, such as the neutral protease chymase and TNF- α , also have pro-apoptotic properties.⁹⁵⁻⁹⁸ Chymase has been shown to induce SMC apoptosis in vitro⁹⁵ by a mechanism involving proteolytic degradation of the PCM component, fibronectin, with subsequent disruption of focal adhesions (Figure 2).⁹⁹ The isolated chymase-produced fibronectin fragments are capable of inducing SMC apoptosis in a similar manner as disintegrins, a family of low-molecular-weight arginine-glycine-aspartate-containing peptides inducing EC apoptosis.¹⁰⁰ The disinte-

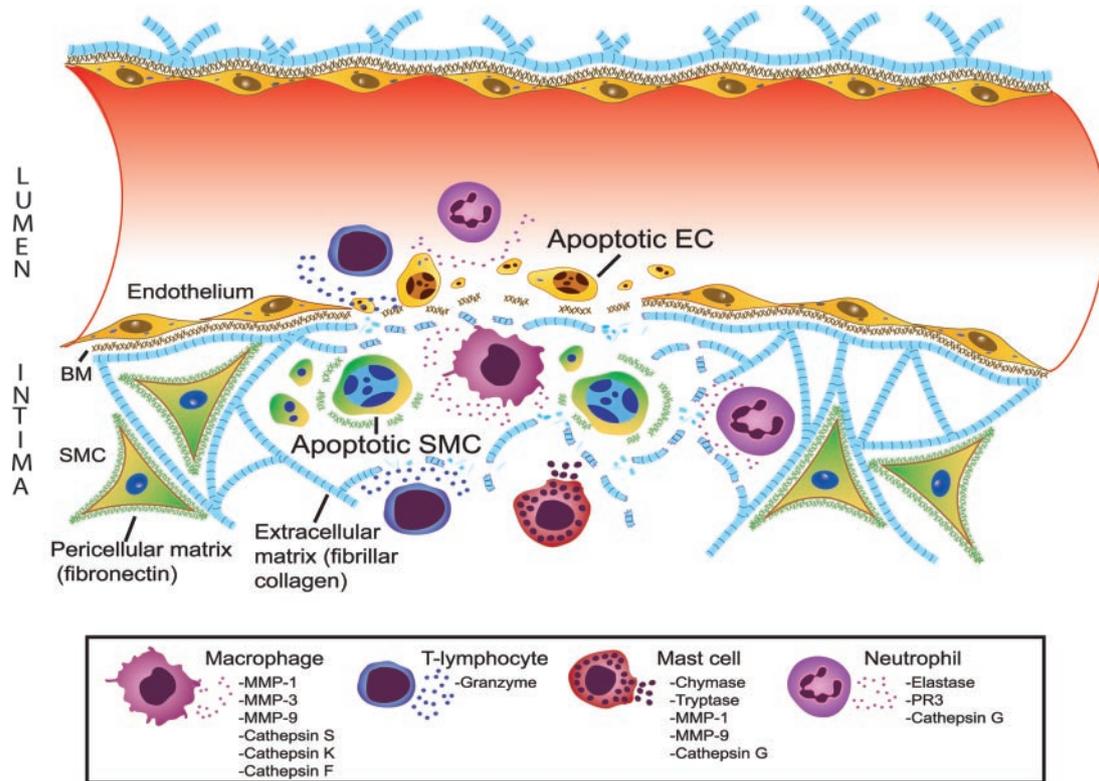


Figure 3. Schematic picture depicting a role for infiltrating inflammatory cells in the erosion and rupture of a coronary atherosclerotic plaque. The infiltrating inflammatory cells, notably macrophages, T-lymphocytes, and mast cells, once activated, secrete a variety of proteases capable of inducing local degradation of the pericellular and extracellular components necessary for cell survival and plaque stability. In addition, in the coronary circulation, T-lymphocytes and neutrophils appear to be activated and to secrete agents capable of damaging the coronary endothelium. Neutrophils may also enter an eroded or ruptured atherosclerotic plaque and further contribute to its pathological remodeling. The scenario provides a mechanistic explanation for the inflammation-dependent plaque weakening and rupture, caused by proteolytic degradation of both PCM and ECM of the plaque. Note: in the boxed area, the proteinases secreted by the infiltrating inflammatory cells and capable of degrading the pericellular and extracellular components are listed together with the respective parent inflammatory cell.

grins are also able to inhibit angiogenesis by provoking endothelial cell detachment and apoptosis.¹⁰⁰ Moreover, in coculture studies, mast cell activation has been shown to induce EC apoptosis by a mechanism that involves TNF- α -mediated downregulation of bcl-2 expression.⁹⁶ The mast cell-mediated pro-apoptotic effect seems to be purely paracrine, because the activated mast cells themselves survive the process of degranulation by overexpressing the prosurvival bcl-2 homologue A1.¹⁰¹

Neutrophils in Plaque Erosion and Rupture

In contrast to macrophages, T-lymphocytes, and mast cells, the presence of neutrophils in unstable lesions have received only little attention, despite the fact that neutrophils are the first-appearing phagocytic cells in acute inflammatory responses to tissue injury, and they have also been identified at sites of plaque rupture.¹⁰² Recent evidence shows that neutrophil infiltrates are present in culprit lesions in acute coronary syndromes,¹⁰³ and accumulation of neutrophils occurs at the site of endothelial denudation almost instantly after coronary artery bypass grafting.¹⁰⁴ Clinical studies have also shown that neutrophils become activated in the coronary circulation of patients with unstable angina pectoris and acute myocardial infarction.^{105–107}

Activated neutrophils are known to release a variety of enzymes, such as elastase, myeloperoxidase, and neutrophil

proteinase 3 (PR3), which are all capable of degrading ECM and/or inducing apoptosis. In particular, neutrophil elastase has been shown to mediate degradation of endothelial basement membrane constituents and to cause endothelial apoptosis.¹⁰⁸ PR3 is a caspase-like protease that may enter the endothelial cells and then cleave NF- κ B, inducing sustained activation of JNK and inactivation of the major cell cycle inhibitor p21. Thus, PR3, as an exogenous caspase-like molecule, can sidestep intracellular caspase functions at sites of inflammation.¹⁰⁹ It was also recently shown that PMNs contain perforin and granzyme B, the 2 molecules known as the cytotoxic entity of NK cells and cytotoxic T-lymphocytes.¹¹⁰ Thus, neutrophil activation with secretion of proteolytic enzymes may be another potential ECM- and PCM-degrading component in acute coronary syndromes. However, because neutrophils are seldom seen in stable plaques with intact endothelium, their presence in unstable plaques of patients with acute coronary syndromes may be a secondary result of the superimposed “acute” inflammation that occurs only after a mural thrombus has developed at the site of erosion or rupture. Although neutrophils appear to become activated in the coronary circulation and attached to plaque surface only after erosion or rupture, their vast array of proinflammatory components, once released, may play a pathogenic role in the propagation of ACSs and therefore

significantly affect the clinical outcome of an acute coronary event.

Conclusions

A novel link between local inflammation in coronary plaques and acute coronary syndromes is emerging. The infiltrating inflammatory cells, such as macrophages, lymphocytes, and mast cells, have been found to secrete a variety of proteases capable of degrading matrix components, followed by death of intimal SMCs and ECs, and therefore provide a mechanistic explanation for the inflammation-dependent plaque weakening and rupture (Figure 3). In addition, in the coronary circulation, T-lymphocytes and neutrophils appear to be activated and to secrete agents capable of damaging the coronary endothelium. Thus, the vulnerability of an atherosclerotic plaque critically depends not only on the integrity of the ECM components, such as fibrillar collagen, but also on the integrity of the PCM surrounding plaque cells and ultimately on the survival of the plaque cells themselves. However, for this hypothesis to become useful in understanding the conversion of a clinically silent plaque into a dangerous and potentially killing plaque, better animal models of plaque erosion and rupture need to be established. Hopefully then, comparison of the results from human pathology, from in vivo animal studies, and from cell culture experiments will pave the way to the solution of the riddle of the vulnerable plaque. Ultimately, the question "to erode or rupture or not" may critically depend on the proteolytic mechanisms governing the balance between cell survival and death in the atherosclerotic plaque.

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References

- Geng YJ, Libby P. Progression of atheroma: a struggle between death and procreation. *Arterioscler Thromb Vasc Biol.* 2002;22:1370–1380.
- Michel JP. Anoikis in the Cardiovascular System: Known and Unknown Extracellular Mediators. *Arterioscler Thromb Vasc Biol.* 2003;23:2146–2154.
- Ross R, Glomset J, Harker L. Response to injury and atherogenesis. *Am J Pathol.* 1977;86:675–684.
- Thomas WA, Kim DN. Biology of disease. Atherosclerosis as a hyperplastic and/or neoplastic process. *Lab Invest.* 1983;48:245–255.
- Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban HE, Perin M, Colombo A, Schuler G, Barragan P, Guagliumi G, Molnar F, Falotico R. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med.* 2002;346:1773–1780.
- Gordon D, Reidy MA, Benditt EP, Schwartz SM. Cell proliferation in human coronary arteries. *Proc Natl Acad Sci U S A.* 1990;87:4600–4604.
- Kockx MM, De Meyer GRY, Muhring J, Jacob W, Bult H, Herman AG. Apoptosis and related proteins in different stages of human atherosclerotic plaques. *Circulation.* 1998;97:2307–2315.
- Pickering JG, Weir L, Jekanowski J, Kearney MA, Isner JM. Proliferative activity in peripheral and coronary atherosclerotic plaque among patients undergoing percutaneous revascularization. *J Clin Invest.* 1993;91:1469–1480.
- Libby P. Molecular bases of the acute coronary syndromes. *Circulation.* 1995;91:2844–2850.
- Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Ann Rev Biochem.* 1983;52:223–261.
- Steinberg D. Lipoproteins and the pathogenesis of atherosclerosis. *Circulation.* 1987;76:508–514.
- Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P, Pasterkamp G, Fayad Z, Stone PH, Waxman S, Raggi P, Madjid M, Zarrabi A, Burke A, Yuan C, Fitzgerald PJ, Siscovick DS, de Korte CL, Aikawa M, Juhani Airaksinen KE, Assmann G, Becker CR, Chesebro JH, Farb A, Galis ZS, Jackson C, Jang IK, Koenig W, Lodder RA, March K, Demirovic J, Navab M, Priori SG, Rekhater MD, Bahr R, Grundy SM, Mehran R, Colombo A, Boerwinkle E, Ballantyne C, Insull W, Jr., Schwartz RS, Vogel R, Serruys PW, Hansson GK, Faxon DP, Kaul S, Drexler H, Greenland P, Muller JE, Virmani R, Ridker PM, Zipes DP, Shah PK, Willerson JT. From Vulnerable Plaque to Vulnerable Patient: A Call for New Definitions and Risk Assessment Strategies: Part I. *Circulation.* 2003;108:1664–1672.
- Falk E, Shah PK, Fuster V. Coronary Plaque Disruption. *Circulation.* 1995;92:657–671.
- Passerini AG, Polacek DC, Shi C, Francesco NM, Manduchi E, Grant GR, Pritchard WF, Powell S, Chang GY, Stoeckert CJ, Jr., Davies PF. Coexisting proinflammatory and antioxidative endothelial transcription profiles in a disturbed flow region of the adult porcine aorta. *PNAS.* 2004;101:2482–2487.
- Berk BC, Min W, Yan C, Surapitsitach J, Liu Y, Hoefen R. Atheroprotective mechanisms activated by fluid shear stress in endothelial cells. *Drug News Perspect.* 2002;15:133–139.
- Cullen P, Baetta R, Bellosa S, Bernini F, Chinetti G, Cignarella A, von Eckardstein A, Exley A, Goddard M, Hofker M, Hurt-Camejo E, Kanters E, Kovanen P, Lorkowski S, McPheat W, Pentikainen M, Rauterberg J, Ritchie A, Staels B, Weikamp B, de Winther M, for the MAFAPS Consortium. Rupture of the Atherosclerotic Plaque: Does a Good Animal Model Exist? *Arterioscler Thromb Vasc Biol* 2003;23:535–542.
- Ball RY, Stowers EC, Burton JH, Cary NRB, Skepper JN, Mitchinson MJ. Evidence that the death of macrophage foam cells contributes to the lipid core of atheroma. *Atherosclerosis.* 1995;114:45–54.
- Björkerud S, Björkerud B. Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumulation of gruel and plaque instability. *Am J Pathol.* 1996;149:367–380.
- Flugelman MY, Virmani R, Correa R, Yu ZX, Farb A, Leon MB, Elami A, Epstein SE. Smooth muscle cell abundance and fibroblast growth factors in coronary lesions of patients with nonfatal unstable angina. A clue to the mechanism of transformation from the stable to the unstable clinical state. *Circulation.* 1993;88:2493–2500.
- Littlewood TD, Bennett MR. Apoptotic cell death in atherosclerosis. *Curr Opin Lipidol.* 2003;14:469–475.
- Mallat Z, Tedgui A. Current perspective on the role of apoptosis in atherothrombotic disease. *Circ Res.* 2001;88:998–1003.
- Arbustini E, Dal Bello B, Morbini P, Burke AP, Bocciaelli M, Specchia G, Virmani R. Plaque erosion is a major substrate for coronary thrombosis in acute myocardial infarction. *Heart.* 1999;82:269–272.
- Kataoka H, Kume N, Miyamoto S, Minami M, Morimoto M, Hayashida K, Hashimoto N, Kita T. Oxidized LDL modulates Bax/Bcl-2 through the lectin-like Ox-LDL receptor-1 in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol.* 2001;21:955–960.
- Sukhanov S, Hua SY, Delafontaine P. Global analysis of differentially expressed genes in oxidized LDL-treated human aortic smooth muscle cells. *Biochem Biophys Res Commun.* 2003;306:443–449.
- Patel VA, Zhang QJ, Siddle K, Soos MA, Goddard M, Weissberg PL, Bennett MR. Defect in insulin-like growth factor-1 survival mechanism in atherosclerotic plaque-derived vascular smooth muscle cells is mediated by reduced surface binding and signaling. *Circ Res.* 2001;88:895–902.
- Dimmeler S, Zeiher AM. Reactive oxygen species and vascular cell apoptosis in response to angiotensin II and pro-atherosclerotic factors. *Regul Pept.* 2000;90:19–25.
- Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol.* 1994;124:619–626.
- Meredith JE, Jr., Fazeli B, Schwartz MA. The extracellular matrix as a cell survival factor. *Mol Biol Cell.* 1993;4:953–961.
- Frisch SM, Ruoslahti E. Integrins and anoikis. *Curr Opin Cell Biol.* 1997;9:701–706.

30. Dimmeler S, Haendeler J, Zeiher AM. Regulation of endothelial cell apoptosis in atherothrombosis. *Curr Opin Lipidol*. 2002;13:531–536.
31. Bosman FT, Stamenkovic I. Functional structure and composition of the extracellular matrix. *J Pathol*. 2003;200:423–428.
32. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell*. 1992;69:11–25.
33. Ruoslahti E. Integrins. *J Clin Invest*. 1991;87:1–5.
34. Giancotti FG, Ruoslahti E. Integrin signaling. *Science*. 1999;285:1028–1032.
35. Stupack DG, Cheresh DA. Get a ligand, get a life: integrins, signaling and cell survival. *J Cell Sci*. 2002;115:3729–3738.
36. Matter ML, Ruoslahti E. A signaling pathway from the $\alpha 5\beta 1$ and $\alpha(v)\beta 3$ integrins that elevates bcl-2 transcription. *J Biol Chem*. 2001;276:27757–27763.
37. Sonoda Y, Matsumoto Y, Funakoshi M, Yamamoto D, Hanks SK, Kasahara T. Anti-apoptotic role of focal adhesion kinase (FAK). Induction of inhibitor-of-apoptosis proteins and apoptosis suppression by the overexpression of FAK in a human leukemic cell line, HL-60. *J Biol Chem*. 2000;275:16309–16315.
38. Borges E, Jan Y, Ruoslahti E. Platelet-derived Growth Factor Receptor beta and Vascular Endothelial Growth Factor Receptor 2 Bind to the beta 3 Integrin through Its Extracellular Domain. *J Biol Chem*. 2000;275:39867–39873.
39. Eliceiri BP. Integrin and Growth Factor Receptor Crosstalk. *Circ Res*. 2001;89:1104–1110.
40. Lee RT, Libby P. The unstable atheroma. *Arterioscler Thromb Vasc Biol*. 1997;17:1859–1867.
41. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med*. 2000;6:389–395.
42. Dollery CM, Owen CA, Sukhova GK, Krettek A, Shapiro SD, Libby P. Neutrophil elastase in human atherosclerotic plaques: production by macrophages. *Circulation*. 2003;107:2829–2836.
43. Vartio T, Seppä H, Vaheri A. Susceptibility of soluble and matrix fibronectins to degradation by tissue proteinases, mast cell chymase and cathepsin G. *J Biol Chem*. 1981;256:471–477.
44. Lohi J, Harvima I, Keski-Oja J. Pericellular substrates of human mast cell tryptase: 72,000 dalton gelatinase and fibronectin. *J Cell Biochem*. 1992;50:337–349.
45. Heeneman S, Cleutjens JP, Faber BC, Creemers EE, van Suylen RJ, Lutgens E, Cleutjens KB, Daemen MJ. The dynamic extracellular matrix: intervention strategies during heart failure and atherosclerosis. *J Pathol*. 2003;200:516–525.
46. Kanse SM, Benzakour O, Kanthou C, Kost C, Lijnen HR, Preissner KT. Induction of vascular SMC proliferation by urokinase indicates a novel mechanism of action in vasoproliferative disorders. *Arterioscler Thromb Vasc Biol*. 1997;17:2848–2854.
47. Meilhac O, Ho-Tin-Noe B, Houard X, Philippe M, Michel JB, Angles-Cano E. Pericellular plasmin induces smooth muscle cell anoikis. *FASEB J*. 2003;17:1301–1303.
48. Friedl P, Wolf K. Proteolytic and non-proteolytic migration of tumor cells and leucocytes. *Biochem Soc Symp*. 2003;70:277–285.
49. Moreau M, Brocheriou I, Petit L, Ninio E, Chapman MJ, Rouis M. Interleukin (IL)-8 mediates downregulation of tissue inhibitor of metalloproteinase-1 expression in cholesterol-loaded human macrophages: relevance to stability of atherosclerotic plaque. *Circulation*. 1999;99:420–426.
50. Xu XP, Meisel SR, Ong JM, Kaul S, Cercece B, Rajavashisth TB, Sharifi B, Shah PK. Oxidized low-density lipoprotein regulates matrix metalloproteinase-9 and its tissue inhibitor in human monocyte-derived macrophages. *Circulation*. 1999;99:993–998.
51. Lijnen HR. Extracellular proteolysis in the development and progression of atherosclerosis. *Biochem Soc Trans*. 2002;30:163–167.
52. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest*. 1994;94:2493–2503.
53. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res*. 2002;90:251–262.
54. Boyle JJ, Bowyer DE, Weissberg PL, Bennett MR. Human blood-derived macrophages induce apoptosis in human plaque-derived vascular smooth muscle cells by Fas-ligand/Fas interactions. *Arterioscler Thromb Vasc Biol*. 2001;21:1402–1407.
55. Hegyi L, Skepper JN, Cary NRB, Mitchinson MJ. Foam cell apoptosis and the development of the lipid core of human atherosclerosis. *J Pathol*. 1996;180:423–429.
56. Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, Sweeney M, Rong JX, Kuriakose G, Fisher EA, Marks AR, Ron D, Tabas I. The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nat Cell Biol*. 2003;5:781–792.
57. Li W, Dalen H, Eaton JW, Yuan XM. Apoptotic death of inflammatory cells in human atheroma. *Arterioscler Thromb Vasc Biol*. 2001;21:1124–1130.
58. Nhan TQ, Liles WC, Chait A, Fallon JT, Schwartz SM. The p17 Cleaved Form of Caspase-3 Is Present Within Viable Macrophages In Vitro and in Atherosclerotic Plaque. *Arterioscler Thromb Vasc Biol*. 2003;23:1276–1282.
59. Li DY, Chen HJ, Staples ED, Ozaki K, Annex B, Singh BK, Vermani R, Mehta JL. Oxidized low-density lipoprotein receptor LOX-1 and apoptosis in human atherosclerotic lesions. *J Cardiovasc Pharmacol Ther*. 2002;7:147–153.
60. Blanc J, Alves-Guerra MC, Esposito B, Rousset S, Gourdy P, Ricquier D, Tedgui A, Miroux B, Mallat Z. Protective role of uncoupling protein 2 in atherosclerosis. *Circulation*. 2003;107:388–390.
61. Martinet W, Knaapen MW, De Meyer GR, Herman AG, Kockx MM. Overexpression of the anti-apoptotic caspase-2 short isoform in macrophage-derived foam cells of human atherosclerotic plaques. *Am J Pathol*. 2003;162:731–736.
62. Sukhova GK, Shi GP, Simon DI, Chapman HA, Libby P. Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. *J Clin Invest*. 1998;102:576–583.
63. Jormsjö S, Wuttge DM, Sirsjö A, Whatling C, Hamsten A, Stemme S, Eriksson P. Differential expression of cysteine and aspartic proteases during progression of atherosclerosis in apolipoprotein E-deficient mice. *Am J Pathol*. 2002;161:939–945.
64. Öörni K, Sneek M, Brömme D, Pentikäinen MO, Lindstedt KA, Mäyränpää M, Aitio H, Kovanen PT. Cysteine protease cathepsin F is expressed in human atherosclerotic lesions, is secreted by cultured macrophages, and modifies LDL particles in vitro. *J Biol Chem*. 2004; Jun 7. Epub ahead of print.
65. Shi GP, Sukhova GK, Grubb A, Ducharme A, Rhode LH, Lee RT, Ridker PM, Libby P, Chapman HA. Cystatin C deficiency in human atherosclerosis and aortic aneurysms. *J Clin Invest*. 1999;104:1191–1197.
66. Edwards KM, Kam CM, Powers JC, Trapani JA. The Human Cytotoxic T Cell Granule Serine Protease Granzyme H Has Chymotrypsin-like (Chymase) Activity and Is Taken Up into Cytoplasmic Vesicles Reminiscent of Granzyme B-containing Endosomes. *J Biol Chem*. 1999;274:30468–30473.
67. Schonbeck U, Mach F, Sukhova GK, Murphy C, Bonnefoy JY, Fabunmi RP, Libby P. Regulation of matrix metalloproteinase expression in human vascular smooth muscle cells by T lymphocytes: a role for CD40 signaling in plaque rupture? *Circ Res*. 1997;81:448–454.
68. Seko Y, Sato O, Takagi A, Tada Y, Matsuo H, Yagita H, Okumura K, Yazaki Y. Perforin-secreting killer cell infiltration in the aortic tissue of patients with atherosclerotic aortic aneurysm. *Jpn Circ J*. 1997;61:965–970.
69. Nakajima T, Schulte S, Warrington KJ, Kopecky SL, Frye RL, Goronzy JJ, Weyand CM. T-cell-mediated lysis of endothelial cells in acute coronary syndromes. *Circulation*. 2002;105:570–575.
70. Hansson GK. Immune Mechanisms in Atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2001;21:1876–1890.
71. Inagaki Y, Yamagishi S, Amano S, Okamoto T, Koga K, Makita Z. INF- γ -induced apoptosis and activation of THP-1 macrophages. *Life Sci*. 2002;71:2499–2508.
72. Aukrust P, Muller F, Ueland T, Berget T, Aaser E, Brunsvig A, Solum NO, Forfang K, Froland SS, Gullestad L. Enhanced Levels of Soluble and Membrane-Bound CD40 Ligand in Patients With Unstable Angina: Possible Reflection of T Lymphocyte and Platelet Involvement in the Pathogenesis of Acute Coronary Syndromes. *Circulation*. 1999;100:614–620.
73. Geng YJ, Henderson LE, Levesque EB, Muszynski M, Libby P. Fas is expressed in human atherosclerotic intima and promotes apoptosis of cytokine-primed human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 1997;17:2200–2208.
74. Alcouffe J, Therville N, Segui B, Nazzari D, Blaes N, Salvayre R, Thomsen M, Benoist H. Expression of membrane-bound and soluble

- FasL in Fas- and FADD-dependent T lymphocyte apoptosis induced by mildly oxidized LDL. *FASEB J*. 2003;18:122–124.
75. Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *J Allergy Clin Immunol*. 2000;105:847–859.
 76. Galli SJ. New concepts about the mast cell. *N Engl J Med*. 1993;328:257–265.
 77. Kaartinen M, Penttilä A, Kovanen PT. Accumulation of activated mast cells in the shoulder region of human coronary atheroma, the predilection site of atheromatous rupture. *Circulation*. 1994;90:1669–1678.
 78. Kovanen PT. Role of mast cells in atherosclerosis. In: Marone G, ed. *Human basophils and mast cells: clinical aspects*. Basel: S. Karger; 1995:132–170.
 79. Kelley JL, Chi DS, Abou-Auda W, Smith JK, Krishnaswamy G. The molecular role of mast cells in atherosclerotic cardiovascular disease. *Mol Med Today*. 2000;6:304–308.
 80. Leskinen MJ, Kovanen PT, Lindstedt KA. Regulation of smooth muscle cell growth, function and death in vitro by activated mast cells—a potential mechanism for the weakening and rupture of atherosclerotic plaques. *Biochem Pharmacol*. 2003;66:1493–1498.
 81. Schwartz LB, Austen KF. Structure and Function of the Chemical Mediators of Mast Cells. In: Ishizaka K, ed. *Mast cell activation and mediator release*. Basel: S. Karger; 1984:271–321.
 82. Compton SJ, Cairns JA, Holgate ST, Walls AF. Human mast cell tryptase stimulates the release of an IL-8-dependent neutrophil chemotactic activity from human umbilical vein endothelial cells (HUVEC). *Clin Exp Immunol*. 2000;121:31–36.
 83. Pober JS, Gimbrone MA, Jr., Lapierre LA, Mendrick DL, Fiers W, Rothlein R, Springer TA. Overlapping patterns of activation of human endothelial cells by IL 1, tumor necrosis factor, and immune INF. *J Immunol*. 1986;137:1893–1896.
 84. Burns AR, Bowden RA, Abe Y, Walker DC, Simon SI, Entman ML, Smith CW. P-selectin mediates neutrophil adhesion to endothelial cell borders. *J Leukoc Biol*. 1999;65:299–306.
 85. Gordon JR. TGF β 1 and TNF α secreted by mast cells stimulated via the Fc ϵ RI activate fibroblasts for high-level production of monocyte chemoattractant protein-1 (MCP-1). *Cell Immunol*. 2000;201:42–49.
 86. Kaartinen M, Penttilä A, Kovanen PT. Mast cells in rupture-prone areas of human coronary atheromas produce and store TNF- α . *Circulation*. 1996;94:2787–2792.
 87. Saren P, Welgus HG, Kovanen PT. TNF- α and IL-1 β selectively induce expression of 92-kDa gelatinase by human macrophages. *J Immunol*. 1996;157:4159–4165.
 88. Baram D, Vaday GG, Salamon P, Drucker I, Hershkoviz R, Mekori YA. Human mast cells release metalloproteinase-9 on contact with activated T cells: juxtacrine regulation by TNF- α . *J Immunol*. 2001;167:4008–4016.
 89. Di Girolamo N, Wakefield D. In vitro and in vivo expression of interstitial collagenase/MMP-1 by human mast cells. *Dev Immunol*. 2000;7:131–142.
 90. Nikkari ST, O'Brien KD, Ferguson M, Hatsukami T, Welgus HG, Alpers CE, Clowes AW. Interstitial collagenase (MMP-1) expression in human carotid atherosclerosis. *Circulation*. 1995;92:1393–1398.
 91. Saarinen J, Kalkkinen N, Welgus HG, Kovanen PT. Activation of human interstitial procollagenase through direct cleavage of the Leu⁸³-Thr⁸⁴ bond by mast cell chymase. *J Biol Chem*. 1994;269:18134–18140.
 92. Gruber BL, Marchese MJ, Suzuki K, Schwartz LB, Okada Y, Nagase H, Ramamurthy NS. Synovial procollagenase activation by human mast cell tryptase dependence upon matrix metalloproteinase 3 activation. *J Clin Invest*. 1989;84:1657–1662.
 93. Wang Y, Shiota N, Leskinen MJ, Lindstedt KA, Kovanen PT. Mast cell chymase inhibits smooth muscle cell growth and collagen expression in vitro: transforming growth factor- β -dependent and - independent effects. *Arterioscler Thromb Vasc Biol*. 2001;21:1928–1933.
 94. Wang Y, Kovanen PT. Heparin proteoglycans released from rat serosal mast cells inhibit proliferation of rat aortic smooth muscle cells in culture. *Circ Res*. 1999;84:74–83.
 95. Leskinen M, Wang Y, Leszczynski D, Lindstedt KA, Kovanen PT. Mast cell chymase induces apoptosis of vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 2001;21:516–522.
 96. Lätti S, Leskinen M, Shiota N, Wang Y, Kovanen PT, Lindstedt KA. Mast cell-mediated apoptosis of endothelial cells in vitro: A paracrine mechanism involving TNF- α -mediated down-regulation of bcl-2 expression. *J Cell Physiol*. 2003;195:130–138.
 97. Wallach D. Cell death induction by TNF: a matter of self control. *Trends Biochem Sci*. 1997;22:107–109.
 98. Hara M, Matsumori A, Ono K, Kido H, Hwang MW, Miyamoto T, Iwasaki A, Okada M, Nakatani K, Sasayama S. Mast cells cause apoptosis of cardiomyocytes and proliferation of other intramyocardial cells in vitro. *Circulation*. 1999;100:1443–1449.
 99. Leskinen MJ, Lindstedt KA, Wang Y, Kovanen PT. Mast cell chymase induces smooth muscle cell apoptosis by a mechanism involving fibronectin degradation and disruption of focal adhesions. *Arterioscler Thromb Vasc Biol*. 2003;23:238–243.
 100. Wu WB, Peng HC, Huang TF. Disintegrin causes proteolysis of β -catenin and apoptosis of endothelial cells. Involvement of cell-cell and cell-ECM interactions in regulating cell viability. *Exp Cell Res*. 2003;286:115–127.
 101. Karsan A, Yee E, Harlan JM. Endothelial cell death induced by tumor necrosis factor- α is inhibited by the Bcl-2 family member, A1. *J Biol Chem*. 1996;271:27201–27204.
 102. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation*. 1994;89:36–44.
 103. Naruko T, Ueda M, Haze K, van der Wal AC, van der Loos CM, Itoh A, Komatsu R, Ikura Y, Ogami M, Shimada Y, Ehara S, Yoshizawa M, Takeuchi K, Yoshikawa J, Becker AE. Neutrophil infiltration of culprit lesions in acute coronary syndromes. *Circulation*. 2002;106:2894–2900.
 104. Sasaki Y, Suehiro S, Becker AE, Kinoshita H, Ueda M. Role of endothelial cell denudation and smooth muscle cell dedifferentiation in neointimal formation of human vein grafts after coronary artery bypass grafting: therapeutic implications. *Heart*. 2000;83:69–75.
 105. Mehta J, Dinerman J, Mehta P, Saldeen TG, Lawson D, Donnelly WH, Wallin R. Neutrophil function in ischemic heart disease. *Circulation*. 1989;79:549–556.
 106. Dinerman JL, Mehta JL, Saldeen TG, Emerson S, Wallin R, Davda R, Davidson A. Increased neutrophil elastase release in unstable angina pectoris and acute myocardial infarction. *J Am Coll Cardiol*. 1990;15:1559–1563.
 107. Biasucci LM, D'Onofrio G, Liuzzo G, Zini G, Monaco C, Caligiuri G, Tommasi M, Rebuzzi AG, Maseri A. Intracellular neutrophil myeloperoxidase is reduced in unstable angina and acute myocardial infarction, but its reduction is not related to ischemia. *J Am Coll Cardiol*. 1996;27:611–616.
 108. Yang JJ, Kettritz R, Falk RJ, Jennette JC, Gaido ML. Apoptosis of endothelial cells induced by the neutrophil serine proteases proteinase 3 and elastase. *Am J Pathol*. 1996;149:1617–1626.
 109. Pendergraft WF, Rudolph EH, Falk RJ, Jahn JE, Grimmmer M, Hengst L, Jennette JC, Preston GA. Proteinase 3 sidesteps caspases and cleaves p21Waf1/Cip1/Sdi1 to induce endothelial cell apoptosis. *Kidney Int*. 2004;65:75–84.
 110. Wagner C, Iking-Konert C, Deneffle B, Stegmaier S, Hug F, Hansch GM, Granzyme B and perforin: constitutive expression in human polymorphonuclear neutrophils. *Blood*. 2004;103:1099–1104.

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