

Atherothrombosis in Acute Coronary Syndromes:

Mechanisms, Markers, and Mediators of Vulnerability

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Abstract

We review the concept of atherothrombosis and the critically important role of inflammation in the development of acute coronary syndromes (ACS). Inflammation is now known to be a major driving force underlying the initiation of coronary plaques, their unstable progression, and eventual disruption, and it also contributes significantly to thrombotic complications that occur in ACS. In addition, we discuss the various local mediators and systemic markers that are involved in the inflammatory process, and review the concepts of “vulnerable plaque,” “vulnerable blood,” “vulnerable myocardium,” and the “vulnerable patient” who is at increased risk for ACS.

Key Words: Inflammation, markers, mediators, vulnerable, acute coronary syndrome.

Introduction

ATHEROTHROMBOSIS is essentially defined as atherosclerosis and its thrombotic complications. With respect to coronary artery disease (CAD), the term implies that there is an interdependent relationship between plaque growth and arterial thrombosis that provides the framework for precipitating an acute coronary syndrome (ACS) (1). Inflammation plays a central role throughout the entire disease progression (2), serving as the engine that runs the atherothrombotic machine. Plaques within the coronary circulation become “high-risk,” “unstable” or “disruption-prone” (“vulnerable plaque”) in response to a wide array of local and systemic influences that are patient-specific. Thrombus formation in association with these lesions may be accelerated or amplified under these same influences (“vulnerable blood”) (3). Similarly, at-risk myocardium that is prone to rhythm disturbances or

subject to ischemic flows is likely to experience dysfunction (“vulnerable myocardium”) (4). Thus, it is not simply that (given ample time) a largely occlusive lesion invariably ruptures and thromboses a coronary vessel. Rather, a combination of risk factors contributes to a vulnerable plaque composition, prothrombotic milieu, and susceptible heart—conditions that strongly favor the clinical manifestation of unstable angina, myocardial infarction, or sudden cardiac death. In order to better predict who is at risk for an acute coronary event (“vulnerable patient”) (4), knowledge of the local and systemic contributors to plaque initiation, progression, disruption, and thrombotic complication is fundamental.

Atherogenesis, Remodeling, and Plaque Disruption

Advances in the understanding of atheromatous coronary disease have focused our attention on the composition of the plaque rather than the degree of stenosis as the major pathophysiologic determinant of disease. Nonstenotic lesions are far more frequent than stenotic plaques, and it has been observed that in up to two-thirds of patients with ACS, relatively small, nonocclusive lesions within a coronary artery may become complicated by thrombus and rapidly progress to total occlu-

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Glossary

ACS = acute coronary syndrome
 ADP = adenosine diphosphate
 Apo(a) = apolipoprotein(a)
 AT₁ = type 1-angiotensin
 CAD = coronary artery disease
 CAM = cell adhesion molecule
 CD40L = CD40 ligand
 CRP = C-reactive protein
 EC = endothelial cell
 ECM = extracellular matrix
 HDL = high-density lipoprotein cholesterol
 IVUS = intravascular ultrasound

LDL = low-density lipoprotein cholesterol
 Lp(a) = lipoprotein(a)
 MMP = matrix metalloproteinase
 PAI-1 = plasminogen-activator inhibitor type-1
 PPAR = peroxisome proliferator-activated receptors
 sCD40L = soluble, biologically active CD40L
 SMC = smooth muscle cell
 TF = tissue factor
 TFPI = TF pathway inhibitor
 TIMP = tissue inhibitors of metalloproteinases
 TNF = tumor necrosis factor

sion (5). In contrast to plaques that form in vascular beds of the brain and periphery, syndrome-producing “culprit” lesions of the coronary arteries do not necessarily create critical degrees of luminal stenosis as they develop. Thus, what should be considered a high-risk lesion may not be apparent with coronary angiography despite the fact that the patient may be at increased risk of experiencing an acute adverse event (6). Identification of individuals who have—or who are likely to generate—vulnerable or unstable plaque phenotypes, in addition to those with pre-existing subocclusive or occlusive disease, represents a shift from traditional strategies of coronary imaging and risk stratification.

The atherosclerotic process occurs within the intima of large- and medium-sized arteries throughout the body and is present as early as the first few years of life (7). Under the influence of a host of behavioral, biochemical, environmental, and genetic factors, plaques (in susceptible individuals) generally progress through various phases of evolution, from stable, asymptomatic, grossly invisible lesions to unstable, high-risk atheromas. The earliest changes begin within the endothelium, where activated endothelial cells (ECs) recruit monocytes and T lymphocytes to the vessel wall (7). Endothelial dysfunction drives this process, which is marked by EC expression of leukocyte and vascular-cell adhesion molecules (CAMs) and increased endothelial permeability to lipoproteins, leukocytes, and other inflammatory mediators (8). Increasing numbers of atherogenic lipoproteins and T lymphocytes within the intima stimulate monocytes to become macrophages and then macrophage-derived, lipid-laden foam cells as they ingest modified lipoproteins. Smooth muscle cells (SMCs) migrate and proliferate, leukocyte recruitment amplifies, and platelet aggregates adhere to injured endothelium in response to a variety of

inflammatory mediators secreted by ECs, activated leukocytes, SMCs, and platelets (9). These lesions represent what are commonly referred to as “fatty streaks” and are often present in individuals by early adulthood. With continued progression, these plaques accumulate pools of extracellular lipid deposits that surround increasing numbers of inflammatory cells, SMCs, and connective tissue elements, all of which comprise a pro-atherogenic, prothrombotic, dynamic environment known as the “extracellular matrix” (ECM) (10). In response to a variety of cytokines and growth factors (e.g., transforming growth factor- β [TGF- β]), a fibrous cap, composed primarily of SMCs and collagen, develops around the expanding lipid core, walling it off from the lumen (11). The atheromatous core accumulates larger, more confluent amounts of extracellular lipids along with inflammatory mediators (e.g., interferon- γ) and proteolytic enzymes (e.g., matrix metalloproteinases [MMPs]) that contribute to thinning of the now well-established fibrous cap, by digesting its components (12).

Plaque disruption during this phase often results in the formation of mural thrombus, but may quickly progress to total occlusion in the setting of increased blood thrombogenicity (13). Through a continuous process of ECM remodeling, more advanced, complicated lesions develop. These plaques, which often, but do not always, create significant degrees of luminal stenosis, are characterized by a degraded fibrous cap with superimposed organizing thrombus and a well-formed, mostly acellular necrotic core containing oxygen radicals, oxidized lipids, dying foam cells, erythrocyte membranes and apoptotic cellular debris (“thin-cap fibroatheroma”). Given the appropriate stimuli, these high-risk atheromas may progress to largely occlusive and calcified or fibrotic atheromas, which may in turn trigger signs and symp-

toms of angina pectoris and ACS that occur secondary to acute thrombosis or during periods of inadequate collateral/luminal blood flow.

The progression of a fatty streak to a high-risk atheroma occurs through a continuous process of ECM remodeling. Dysregulation of ECM metabolism may result in an accelerated accumulation of lipids and foam cells (14), a net increase in collagen resorption with subsequent weakening of the fibrous cap (15), and compensatory changes in vessel wall architecture leading to ectasia (16). Neovascularization in atherosclerotic arteries introduces fragile intimal microvessels (*vasa vasorum*), which may rupture into the core, resulting in repeated, often subclinical, intraplaque hemorrhage (17). As these clots reorganize and are layered with fibrous tissue, the lesion advances (18). Expansive remodeling of the ECM results in outward growth of the plaque, increasing the circumference of the diseased section of artery and preserving the lumen (19). As the extent of luminal narrowing is inversely proportional to the degree of expansive remodeling (20), relatively non-stenotic segments observed during angiography may harbor advanced, disruption-prone plaques (21) that have been identified as culprit lesions in patients with ACS, using intravascular ultrasound (IVUS) (19). In contrast, constrictive remodeling of plaques, which promotes luminal stenosis, is more commonly associated with stable plaque phenotypes that are evident in patients with stable angina pectoris (22).

Early studies described frank rupture of a plaque as the cause of thrombosis in nearly three-quarters of subjects with sudden cardiac death (23). More recent observations suggest that roughly 60–70% of acute coronary thromboses result from plaque rupture, while as many as 30–40% are associated with superficial erosion of the high-risk plaque (24). Necropsy studies of atheromatous coronary arteries reveal some plaque disruptions to be clinically silent (25). Repetitive cycles of subclinical rupture and healing appear to be a potent stimulus for growth and often result in an accelerated progression toward advanced disease (26).

Thrombosis

Disruption of an atherosclerotic plaque, via rupture or erosion with *in situ* formation of a blood clot that occludes the lumen of a coronary artery, is the pivotal event in the atherothrombotic process leading to ACS. Inflammation plays a key role during thrombogenesis as well, as procoagulant factors within the ECM are exposed to luminal blood

flow at sites where plaque disruption has occurred (27). Stimulated by inflammatory mediators, circulating platelets adhere to damaged endothelium and form aggregates that become enmeshed with fibrin (3). Given the appropriate mixture of disturbed blood flow, inflammation, and thrombogenic potential, occlusive thrombi may trigger ACS even in the absence of visible plaque disruption (28). A growing body of evidence suggests that hyperlipoproteinemia, hypertension, diabetes, hyperhomocysteinemia, cigarette smoking, apoptosis, and the presence of tissue factor (TF), among other conditions, augment the inflammatory and hemodynamic response to vascular injury and feed the coagulation cascade resulting in accelerated thrombogenesis.

Hyperlipidemia

Excess cholesterol deposition within the intimal layer of the vascular wall is a central process in the progression toward unstable atherothrombotic disease (29). Intramural accumulations of extracellular lipids have been observed consistently in a majority of culprit lesions from ACS patients (30). High plasma levels of low-density lipoprotein cholesterol (LDL) and triglycerides (31), and low plasma levels of high-density lipoprotein cholesterol (HDL) have been implicated as major independent cardiovascular risk factors in countless studies, and LDL-lowering is associated with an up to 30% reduction in the incidence of acute coronary events in clinical trials assessing statin pharmacotherapy (32). It has been argued that these lower-density lipoproteins exert pro-atherogenic and prothrombotic influences that contribute to plaque progression, instability, and accelerated thrombogenesis through a variety of interrelated mechanisms.

Structurally and functionally similar to LDL, lipoprotein(a) [Lp(a)] has been implicated by a growing body of research as a “key player” in atherothrombogenesis (33). A major component of Lp(a), apolipoprotein(a) [Apo(a)] is structurally similar to plasminogen and competes with plasminogen by inhibiting its binding to fibrin. Larger amounts of Lp(a) are detected in the lesions of ACS patients when compared with those of stable angina patients, and extensive macrophage colocalization with Lp(a) is observed within culprit lesions, evidence supporting the hypothesized role of Lp(a) as a contributor to both inflammation and plaque disruption (34). Convincing data also suggests that Lp(a) is thrombogenic and promotes coagulation (35), inhibiting fibrinolysis through its interaction with fibrin (36). Altogether, these stud-

ies support a major atherothrombotic function of Lp(a) in the progression toward ACS, yet clinical trials with lipid-lowering agents that specifically target Lp(a) are lacking (37).

Hypertension

The recent literature pertaining to hypertension and atherosclerosis has focused on angiotensin II, a potent vasoconstrictor and principal product of the renin-angiotensin system. Aside from elevating blood pressure, angiotensin II contributes to atherogenesis by inducing endothelial dysfunction (38), stimulating SMC growth (39), attracting monocytes (40), and enhancing macrophage uptake of oxidized lipoproteins (41).

Recent work examining the structure of G protein-coupled type 1-angiotensin (AT₁) receptors suggests that covalent dimerization of AT₁ receptors on the surface of monocytes may account for the pro-inflammatory and atherogenic changes seen in hypertensive patients (42). These observations provide the impetus for the development of monocyte-selective therapeutic agents that specifically target G protein-coupled receptor function in an effort to minimize the atherogenic contribution of hypertension to plaque initiation, progression and acute complication (43).

Diabetes

Diabetes also has a multifactorial role in promoting atherothrombosis. Improved glycemic control has been demonstrated to be associated with a reduction in the risk of cardiovascular disease (44). Hyperinsulinemia alone has been investigated as an independent risk factor, one that increases susceptibility to thrombosis by causing elevated levels of plasminogen-activator inhibitor type-1 (PAI-1) (45, 46). Increased blood thrombogenicity has been demonstrated in diabetics, as has increased platelet reactivity and aggregability (47). The thiazolidinediones have been demonstrated to lower PAI-1 levels, and the improved glycemic control seen with these agents may lead to an overall reduction in atherothrombotic events (48).

Smoking

Cigarette smoking is a well-established risk factor for atherosclerosis and premature CAD. Decades of research have identified several mechanisms that are likely to contribute to accelerated atherothrombogenesis in patients with ACS who smoke. These mainly include EC dysfunction (49), increased oxidative stress (50) and increased blood

thrombogenicity (51), factors that may work alone or in concert with one another to generate a pro-atherogenic, prothrombotic environment conducive to the development of high-risk atherosclerotic disease. Cigarette smoking produces oxygen-derived free radicals and lipid peroxides that impair the endothelium and promote LDL oxidation (52), monocyte adhesion (53), and platelet aggregation. Smoking markedly attenuates endothelium-dependent vasodilatation (54), which suggests that smoking may play a crucial role in the transformation from stable to unstable coronary disease. Additionally, impaired thrombolytic activity is found in smokers (55). These factors are likely to account for the hypercoagulable state and reduced fibrinolytic potential that have been observed in chronic smokers both with and without CAD (56).

Other Agents Implicated in Atherothrombosis

Tissue factor and apoptosis. A glycoprotein abundantly expressed on the surface of a variety of different cell types within atherosclerotic plaques, TF initiates the external clotting cascade and may be a major contributor to increased blood thrombogenicity and accelerated thrombogenesis in ACS patients (57). An animal study reported that monoclonal antibodies directed against TF can decrease thrombogenicity and inhibit both thrombus formation and re-occlusion in diseased arteries (58). Endogenous tissue factor pathway inhibitor (TFPI) appears to be an efficient inhibitor of TF activity, and a reduction in local plaque thrombogenicity has been observed in disrupted human plaques through inhibition of the TF pathway by exogenous TFPI (59).

The contribution of TF to increased blood thrombogenicity seems to be closely linked to apoptosis, or programmed cell death (60), a process that occurs in virtually all cell types present within the fibrous cap and necrotic core of atherosclerotic lesions (61). It has been proposed that apoptotic cells and debris, rich in phosphatidylserine, provide a potent thrombogenic stimulus to a disrupted plaque by activating TF (62). It has also been observed that apoptosis of SMCs occurs to a greater extent within the atherosclerotic plaques of ACS patients as compared with those of stable angina patients (63). Taken all together, these findings highlight an important pro-coagulable, pro-inflammatory contribution of both TF and apoptosis to overall vulnerability in CAD, and the development of therapeutic agents that inhibit the TF pathway at various steps is currently underway.

Homocysteine. More than three decades of research have implicated elevated plasma homocysteine as an independent risk factor for atherosclerotic disease (64). Studies of patients with inborn errors of homocysteine metabolism have identified accelerated CAD that often resulted in a first myocardial infarction by the second decade of life (65). In patients without genetic defects who have elevated plasma homocysteine, the relative risk of CAD was found to be 24 times that of controls (66). Furthermore, a threefold increase in the risk of acute myocardial infarction was reported in a prospective evaluation of men with homocysteine levels just 12% above normal, after accounting for traditional CAD risk factors (67).

Evidence suggests that homocysteine is prothrombotic (68), damages vascular endothelium (69), interferes with endothelium-dependent vasodilation (70), attenuates the effects of NO (71), and increases platelet adhesion (72) and oxidative stress (73). Moreover, a recent study reported an increase in collagen synthesis by vascular SMCs cultured with homocysteine (74), findings which support a pro-atherogenic role of homocysteine.

C-reactive protein. C-reactive protein (CRP), a nonspecific but highly sensitive plasma marker of systemic inflammation, has recently been proposed as an independent risk factor for cardiovascular disease (75, 76). However, it remains unclear whether CRP directly influences plaque initiation and progression (77) or simply represents the inflammatory contribution of another factor.

Plasma elevations of CRP have been reported in patients with acute ischemia and myocardial infarction, and are predictive of the risk of recurrent ischemia among hospitalized patients with unstable angina (78). Recent research suggests that CRP activates ECs and monocytes, and that pharmacologic agents can modulate its effects (79). CRP levels were directly related to the risk of a first myocardial infarction in apparently healthy men given aspirin (80), further evidence supporting a role for anti-inflammatory therapy in ACS prevention. Periodic monitoring of CRP in addition to LDL in patients receiving statin therapy is supported by recent data showing improved clinical outcomes in those with low post-treatment CRP (<2 mg/L) as compared to those with higher levels, regardless of the resultant LDL cholesterol level (81). Future research will hopefully shed more light on the significance of CRP and other inflammatory mediators in the progression toward unstable CAD.

PPARs. Functioning as ligand-receptor transcription factors, peroxisome proliferator-activated receptors (PPAR) belong to a superfamily of nu-

clear receptors that are involved in gene expression throughout the body (82). At the cellular level, these PPAR target genes control a variety of biological processes, including glucose homeostasis, lipid metabolism and inflammation (83), and studies have attempted to establish a link between PPAR signaling and atherothrombotic disease (84, 85). Three subfamilies of PPARs exist: PPAR- α , PPAR- β/δ , and PPAR- γ . PPAR- γ has been the most extensively studied; it has been implicated in the regulatory phase of fatty acid storage within adipose tissue, and is highly expressed by foam cells, SMCs, ECs, mononuclear cells, and macrophages within atherosclerotic plaques (86). Activation of PPAR- γ within the arterial wall has been shown in animal models to lead to a regression of atherosclerotic disease. PPAR- γ activation is likely to result in enhanced fibrinolysis and decreased blood thrombogenicity through its negative effects on PAI-1 and fibrinogen concentrations (87). Finally, PPAR- γ activators have been reported to inhibit the expression of inflammatory mediators including MMPs, CAMs, and cytokines, and may lead to a reduction in plaque inflammation or disruption through one or more of these pathways (88). Notably, fibrate therapy for hyperlipidemia and thiazolidinedione therapy for type 2 diabetes utilize the PPAR- α and PPAR- γ pathways to achieve their respective clinical effects.

Matrix metalloproteinases. MMPs are a diverse family of powerful, zinc-containing enzymes expressed by macrophage-derived foam cells, SMCs and other vascular cells within atherosclerotic lesions (89). It has been previously demonstrated that MMPs are responsible for remodeling of the ECM during all stages of atheromatous development and may directly contribute to fibrous cap weakening and plaque rupture within diseased arteries (90). Together with MMPs, endogenous inhibitors of MMPs—termed “tissue inhibitors of metalloproteinases” (TIMPs)—are expressed within plaques and are capable of inhibiting the matrix-degrading potential of MMPs, presumably by interfering with the cleavage of the zymogen to its active form (91). Current research has focused on MMP inhibition as a potential therapeutic strategy (92), although pharmacologic agents that target specific MMP proenzymes or enhance TIMP activity *in vivo* have yet to be developed (93). Moreover, recent investigations suggest that at least some MMPs are beneficial, participating in the healing of wounds and strengthening of tissues (94, 95). A greater understanding of the pleiotropic effects of MMPs in human atheromas is needed if we are to determine whether therapy aimed at these proteins lessens the risk of unstable CAD.

Key Concepts

1. Atherothrombosis is defined as atherosclerosis and its thrombotic complications.
2. Acute coronary syndromes encompass unstable angina, myocardial infarction, and sudden cardiac death, entities that represent clinical endpoints of the atherothrombotic process.
3. Identification of the vulnerable patient requires careful assessment of plaque vulnerability, blood vulnerability, and myocardial vulnerability in order to more accurately determine an individual's risk of ACS.
4. A growing body of evidence suggests that conditions such as hyperlipoproteinemia, hypertension, diabetes, hyperhomocysteinemia, cigarette smoking, apoptosis, and the presence of TF, among others, augment the inflammatory and hemodynamic response to vascular injury and feed the coagulation cascade resulting in accelerated thrombogenesis.
5. CRP, MMPs, CD40L, and PPARs appear to be intimately involved in plaque progression and may represent therapeutic targets and/or markers of vulnerability within the arena of ACS prevention and treatment.

CD40 ligand. CD40 ligand (CD40L) is an immunoregulatory transmembrane protein that belongs to the tumor necrosis factor (TNF) super family. It is expressed on the surfaces of many cell types, including leukocytes, ECs, SMCs, macrophages, and activated platelets (96). Ligand-receptor binding on these cells triggers the expression and secretion of a variety of pro-inflammatory and procoagulant mediators, including CAMs, cytokines, chemokines, growth factors, MMPs, and TF (96). Recent data suggest that CD40L plays a central role in the inflammatory process that contributes to plaque destabilization in CAD (97), and elevations in soluble, biologically active CD40L (sCD40L) have been demonstrated in the serum of ACS patients (98). Notably, inhibition of both platelet activation and sCD40L release is possible through interference with adenosine diphosphate (ADP)-receptor binding and through GPIIb/IIIa receptor inhibition (99, 100), processes which may exert an overall negative influence on platelet aggregation. This expanding body of evidence lends support to the hypothesis that pharmacologic antagonism of CD40L may help ameliorate the thrombo-inflammatory cascade of events that contributes to plaque progression and thrombosis in patients with ACS (101). However, it remains unclear whether CD40L has a primarily pathogenetic role or is simply a marker of local or systemic inflammation.

Conclusions

In summary, the atherothrombotic process culminating in ACS is both multifactorial and patient-specific. Inflammation appears to play a critical role throughout this process. The concept of the vulnerable patient takes into account contributions made by plaque morphology and composition, blood thrombogenicity and myocardial susceptibility, when determining an individual's risk of an acute

coronary event. Traditional cardiac risk factors that predispose an individual to atheromatous disease are only part of the equation and must be considered within the setting of other local and systemic contributors that may adversely affect coronary plaque progression and thrombotic complication. Our advances in the understanding of the atherothrombotic process will hopefully lead to an earlier detection of vulnerable plaques, a greater predictive value of serum markers, and more specifically targeted pharmacological interventions to find and treat the vulnerable patient at risk of ACS.

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