

Update in Atherothrombotic Disease

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Abstract

Crucial advances in our understanding of the pathogenesis of atherothrombosis, defined as atherosclerosis and its thrombotic complications, have been achieved during the past two decades. The historical hypothesis of pathogenesis ("lipid accumulation") has evolved to integrate several factors contributing to the initiation and evolution of this complex disease. Endothelial dysfunction is considered to be the earliest event in atherogenesis. Inflammation and apoptosis play critical roles in its progression and onset. Tissue factor is postulated to be a central actor in determining plaque thrombogenicity. A hyper-reactive state of the blood ("vulnerable blood") may be responsible for one-third of all the acute coronary syndromes. This review will discuss emerging concepts in the pathogenesis of and therapeutic approaches to atherothrombotic disease.

Key Words: Atherothrombosis, atherosclerosis, antithrombotic therapy, coronary artery disease, acute coronary syndromes, tissue factor, inflammation, apoptosis, endothelial dysfunction.

Atherosclerosis and Atherothrombosis

ATHEROSCLEROSIS, the leading cause of mortality in the Western world, is a diffuse process that starts in early childhood and progresses asymptotically through adult life. Later in life, it is clinically expressed as ischemic coronary syndromes (or coronary artery disease, CAD), stroke, transient ischemic attack (TIA) and peripheral artery disease (PAD). From the clinical point of view, we should envision this disease as the same pathologic entity that affects different vascular beds. From a pathologic point of view, it is characterized by intimal thickening owing to cellular and lipid accumulation that is due to an imbalance in lipid influx and lipid efflux. It affects the intima of large

and medium arteries, including the aorta, as well as the carotid, coronary, and peripheral arteries (1, 2). Secondary changes may occur in the underlying media and adventitia, particularly in advanced disease stages. Fatty streaks have been found in the intima of infants (3). They progress to fibroatheroma by developing a cap of smooth muscle cells and collagen. Atherosclerotic lesions can progress without compromising the lumen because of compensatory vascular enlargement (positive remodeling) (4). Importantly, lipid-rich lesions (culprit lesions) leading to acute coronary syndromes are often mildly stenotic due to the significant positive remodeling, and are therefore not detectable by angiography (5, 6). Plaque disruption and subsequent thrombus formation is responsible for the onset of most acute coronary syndromes and strokes. The magnitude of the thrombotic process triggered upon plaque disruption is modulated by different elements that determine plaque and blood thrombogenicity: local shear rate, tissue factor, apoptotic microparticles, circulating monocytes, etc. The atherosclerotic and thrombotic processes appear to be interdependent and could therefore be integrated under the term "atherothrombosis," a broader term that includes both atherosclerosis and its thrombotic complications.

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Socioeconomic Impact

Coronary artery disease and its thrombotic complications, stroke, transient ischemic attack and peripheral artery disease (PAD) are the leading cause of morbidity and mortality in developed countries. The annual incidence of stroke, TIA and CAD together in the United States is approximately 3.03 million. The annual incidence of emergency department (ED) visits for the evaluation of chest pain is estimated to be approximately 8 million (7). The total cost of CAD in the U.S. for 2002 was \$111.8 billion: direct costs \$58.2 billion, and indirect costs \$53.6 billion (Fig. 1) (8). It can be predicted that the socioeconomic burden of atherothrombosis will continue to increase as the result of several factors: (a) increased survival during acute phases of ischemic disease, transforming acute patients into chronic patients (e.g., ischemic cardiomyopathy, heart failure, post-stroke disability, chronic intermittent claudication); (b) aging of the population; (c) increase in health-related costs; and (d) "Westernization" of the lifestyle in developing countries (e.g., smoking, diabetes, diet, stress, etc.).

"Vulnerable" or "High-Risk" Plaque

Despite a common physiopathologic pathway, atherosclerotic lesions are very heterogeneous, and the "high-risk plaque" of each vascular bed has unique characteristics. Insights into the disease have advanced beyond the notion of progressive occlusion of the coronary artery, to the recognition that plaque disruption and superimposed thrombus formation are the leading causes of acute coronary syndromes and cardiovascular deaths. Therefore, plaque composition (as a determinant of risk of disruption), rather than luminal stenosis, has become the major determinant of this disease (9, 10).



Fig. 1. Cost of coronary heart disease in the United States: year 2002 (8).

Histologically, these rupture-prone (also called vulnerable or high-risk) lesions consist of a large core of extracellular lipid, a high density of macrophages containing lipids, reduced numbers of vascular smooth muscle cells (SMCs), and a thin fibrous cap. Thus, it is not surprising that these plaques are less stable and have a higher propensity to rupture than the fibrous, collagen-rich plaques. Plaque disruption usually occurs at the weakest point ("shoulder"), frequently where the cap is thinnest and most heavily infiltrated with inflammatory cells (11). Once the plaque is disrupted, the highly thrombogenic lipid-rich core, abundant in tissue factor (TF), is exposed to the bloodstream, triggering the formation of a superimposed thrombus that leads to vessel occlusion and subsequent ischemic symptoms distal to the occlusion (12, 13).

In contrast to most of the high-risk coronary plaques, the high-risk carotid plaques are significantly stenotic, heterogeneous, and very fibrous. The disruption is often caused by an intramural hematoma or dissection that probably relates to the systolic stroke of blood against the resistance they offer because of their stenotic condition (14). Although the lipid accumulation in the carotid arteries is quite diffuse, a recent study reported the presence of ruptured lipid-rich plaques in patients with TIA and stroke (15).

Thoracic aorta high-risk plaques frequently contain a high proportion of extracellular lipids and are characterized by a shift toward greater macrophage content relative to SMCs in the cap. At autopsy, aortic plaques from persons who died of ischemic heart disease often have ulceration and mural thrombosis (16). Recent aortic plaque characterization by magnetic resonance imaging confirmed their lipid-rich composition (17).

The plaques at high risk for acute ischemic syndromes of the lower extremities appear to be very stenotic and fibrotic (18). Nonetheless, as distinguished from carotid artery disease, available evidence suggests that in PAD, plaque stenosis associated with hyperthrombogenicity of the systemic blood seems to be a more important contributor to acute ischemic syndromes (sudden ischemic pain, gangrene). This is suggested by the high prevalence of diabetes, cigarette smoking and dyslipidemia (19), which are known to cause a hyperthrombogenic state of the blood (20, 21).

Emerging Concepts in Pathogenesis

Views of the pathophysiology of atherothrombosis have evolved substantially over the past 2

decades. The etiological theory of atherothrombosis has now been widened to include complex biologic processes—such as inflammation, apoptosis, tissue factor (TF) physiology, and the behavioral sciences—in determining the onset of acute ischemic events (Fig. 2).

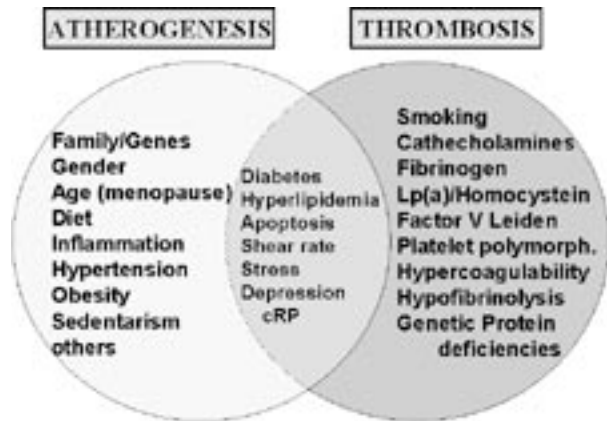


Fig. 2. The circle called atherogenesis includes the most accepted risk factors involved in the development of atherosclerosis. The circle called “Thrombosis” contains a list of prothrombotic factors. In the overlap area are shown the elements involved in both atherosclerosis and thrombosis (i.e., atherothrombosis).
cRP = C-reactive protein

Endothelial Dysfunction

Endothelial dysfunction is a systemic, reversible disorder considered to be the precursor that initiates the atherosclerotic process (22, 23). It is characterized by a reduction of the bioavailability of vasodilators, in particular nitric oxide (NO), and an increase in endothelium-derived contracting factors (Fig. 3). This imbalance leads to an impairment of endothelium-dependent vasodilation, the hallmark of endothelial dysfunction. A dysfunctional endothelium promotes lipid and cell permeability, lipoprotein oxidation, inflammation, SMC proliferation, extracellular matrix deposition or lysis, platelet activation, and thrombus formation (24). The endothelium regulates the production of prothrombotic and antithrombotic factors, growth factors and vasoactive substances. The clear predilection for lesion formation at arterial branching points indicates the importance of local rheologic conditions in atherosclerosis. Furthermore, endothelial cell gene expression is modulated by acute changes in flow profiles (25). A dysfunctional endothelium generates a proatherogenic environment by cre-

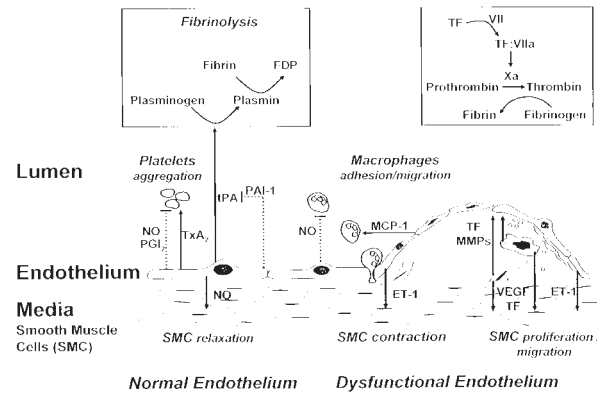


Fig. 3. Impact of endothelial dysfunction on atherogenesis. ET-1 = endothelin-1; FDP = fibrin-degradation products; MCP-1 = monocyte chemoattractant protein-1; MMPs = matrix metalloproteinases; NO = nitric oxide; PAI-1 = plasminogen activator inhibitor; PGI₂ = epoprostenol; SMC = smooth muscle cell; TF = tissue factor; TXA₂ = thromboxane A₂; VEGF = vascular endothelial growth factor

ating a proinflammatory, proliferative and prothrombotic milieu that favors atherogenesis (25). In addition, platelet-derived mediators such as serotonin induce vasoconstriction in the presence of a dysfunctional endothelium (26). Moreover, vasoconstrictor response to these stimuli is increased by endothelin-1 (27), concentrations of which are elevated in the plasma of patients with early and advanced atherosclerosis (28) as well as in culprit lesions (29). Endothelial dysfunction is involved in the recruitment of inflammatory cells into the vessel wall and in the initiation of atherosclerosis. Endothelial cells produce cytokines and express adhesion molecules (e.g., selectins, and vascular and intercellular cell adhesion molecules) and assist leukocytes and other blood-derived cells in “homing” and infiltration. Monocytes migrate into the subendothelium, where they transform into macrophages and modulate inflammatory reactions and the secretion of chemoattractants.

Many of the cardiovascular risk factors, including hyperlipidemia, hypertension, diabetes, and smoking are associated with overproduction of reactive oxygen species or increased oxidative stress, both of which reduce vascular NO bioavailability and promote cellular damage (30). Hence, increased oxidative stress is considered to be a major mechanism involved in the pathogenesis of endothelial dysfunction and may serve as a common pathogenic mechanism of the effect of risk factors on the endothelium (30, 31). In summary, endothelial

dysfunction contributes to enhanced plaque vulnerability, may trigger plaque rupture, and favors thrombus formation; it thus may be viewed as an important causal factor for several aspects of atherothrombotic disease.

Inflammation and Atherothrombosis

There is a growing body of evidence supporting the link between inflammation and atherothrombosis (32). Early in atherogenesis, patches of arterial endothelial cells begin to express on their surface selective adhesion molecules that bind to various classes of leukocytes (Fig. 3). In particular, vascular cell adhesion molecule-1 (VCAM-1) binds precisely to the types of leukocytes found in early human and experimental atheroma, the monocyte and T lymphocyte. Not only does VCAM-1 expression increase on endothelial cells overlying nascent atheroma, but mice genetically engineered to express defective VCAM-1 show interrupted lesion development (33). Once attached to the endothelium, the leukocytes penetrate into the intima. In addition to oxidized low-density lipoprotein, monocyte chemoattractant protein-1 (MCP-1) appears to be responsible for the direct migration of monocytes into the intima at sites of lesion formation (34). Once they are in the arterial wall, the blood-derived inflammatory cells participate in and perpetuate a local inflammatory response. The macrophages express scavenger receptors for modified lipoproteins, permitting them to ingest lipids and become foam cells.

Inflammatory processes not only promote initiation and evolution of atherosclerosis, but also contribute decisively to acute thrombotic complications of atheroma. The activated macrophages abundant in atheroma can produce proteolytic enzymes (e.g., matrix metalloproteinases, MMP) capable of degrading the collagen that lends strength to the plaque's protective fibrous cap, rendering that cap thin, weak, and susceptible to rupture. An overexpression of all three human interstitial collagenases (MMP-1, 8, and 13) has been demonstrated in atheroma (35). In addition, γ -Interferon arising from the activated T lymphocytes in the plaque can halt collagen synthesis by SMCs, limiting its capacity to renew the collagen that reinforces the plaque (36). Macrophages also produce tissue factor, the major procoagulant and trigger to thrombosis found in plaques. Inflammatory mediators regulate tissue factor expression by plaque macrophages, demonstrating an

essential link between arterial inflammation and thrombosis (37).

Elevated values of inflammatory biomarkers, such as C-reactive protein (CRP) (38), interleukin-6 (IL-6) (39), serum amyloid A (40), soluble ICAM-1 (41), and CD40L (42) commonly accompany CAD. Their elevation correlates in some series (43) with adverse prognosis and may reflect the contribution of inflammation as an instigator of atherosclerotic plaque instability.

Tissue Factor

Tissue factor (TF) is known to be a potent initiator of coagulation cascade, and its presence in atherosclerotic plaque suggests its potential role as a key determinant of plaque thrombogenicity (44–46) (Fig. 4). Tissue factor antigen, TF activity, as well as TF mRNA have been identified in different cell types within the atheroma, including endothelial cells (EC), vascular SMCs, and especially cholesterol-enriched macrophages (foam cells) (47). TF antigen is also detected in the extracellular matrix of atherosclerotic plaque and is thought to be derived largely from macrophages present in plaque, with a minor contribution of vascular SMC and EC. Several studies have demonstrated that TF is most abundant in the shoulder region and acellular, lipid-rich core of the plaque (48). Recently, the majority of TF expression within human plaques was found to be in areas of high density of apoptotic cells and microparticles (49). Furthermore, TF activity in the acellular lipid core was found mainly on apoptotic microparticles of monocytic and lym-

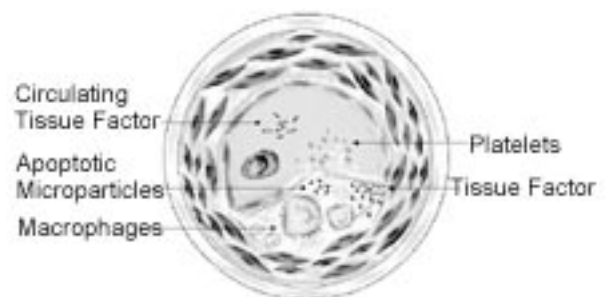


Fig. 4. Disrupted atherosclerotic plaque. The presence of tissue factor and apoptotic microparticles may be responsible for the thrombogenic potential of the disrupted atherosclerotic plaque. Circulating tissue factor is strikingly increased in patients with cardiovascular risk factors; hence it may also play a role in the formation of the thrombus upon atherosclerotic plaque disruption.

phocytic origin (49). These microparticles with procoagulant potential are rich in phosphatidylserine, providing in this way the appropriate phospholipid environment required for TF activity (50). All this evidence highlights the importance of TF in thrombus formation upon plaque disruption; hence, it is attractive to consider TF as a new pharmacologic target.

Apoptosis and Atherothrombosis

The role of apoptosis in atherothrombosis can be described at two separate levels, at the site of the plaque and in the circulation. Apoptosis in atherothrombotic disease involves all cell types (51). Within and around the necrotic core, as well as in the fibrous cap, there is an accumulation of foam cells and nuclear fragments staining positive for terminal deoxynucleotidyl transferase nick end labeling (TUNEL) (52, 53).

Despite the initiation of the cell death process, apoptotic cells are involved in the inflammatory process through further recruitment of other inflammatory cells. For example, the externalization of phosphatidylserine by apoptotic cells, which can be detected with Annexin V (54), has the immunogenic potential to activate neighboring cells for phagocytosis (55).

Mallat and Tedgui (50) reported a clear association between apoptosis and inflammation. They demonstrated coexistence of inflammation and apoptotic cells in areas of plaque rupture that resulted in exposure of a high fraction of apoptotic cells and debris to the circulation (56). It is believed that, besides having immunogenic and inflammatory characteristics, apoptotic cells possess a highly thrombogenic potential (57, 58). Thus, they are postulated as one of the determinants of plaque thrombogenicity.

It has been demonstrated that apoptosis coexists with high levels of TF expression within the atherosclerotic plaque (48). TF is functional on the cell surface and its activity is highly dependent on the presence of phosphatidylserine (PS) (59). Since PS exposure is associated with apoptosis, it has been suggested that apoptosis may be partly responsible for TF activation within plaque. Recently, Hutter et al. (60) demonstrated the co-localization of TF and apoptosis in macrophages within lipid-rich human atherosclerotic plaques and, in addition, showed *in vitro* that a significant percentage of macrophages exposed to oxidized low-density lipoprotein (Ox-LDL) undergoing apoptosis expressed TF. There is further evidence that apoptosis in different cell types promotes procoagulant activity.

Interestingly, the prothrombotic potential of different apoptotic cells is not only confined locally to atherosclerotic plaque, but is also present in circulation. Mallat et al. (61) initially demonstrated the presence of high levels of shed membrane apoptotic particles in extracts from atherosclerotic plaques, but not from the underlying arterial wall. Later, the same group showed the presence of high levels of endothelial membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes, implying that they might participate in the generation and perpetuation of intracoronary thrombi (56). This indicates a major role of apoptosis in atherothrombosis following plaque rupture.

Peroxisomal Proliferator-Activated Receptors

Peroxisomal proliferator-activated receptors (PPARs) are steroid hormone nuclear receptors that act as ligand-activated transcription factors controlling the expression of specific target genes, which in turn regulate a variety of cellular functions (Fig. 5). The subfamily member PPAR- γ plays a central role in adipogenesis and lipid metabolism and is highly expressed in endothelial cells, SMCs, lymphocytes, and

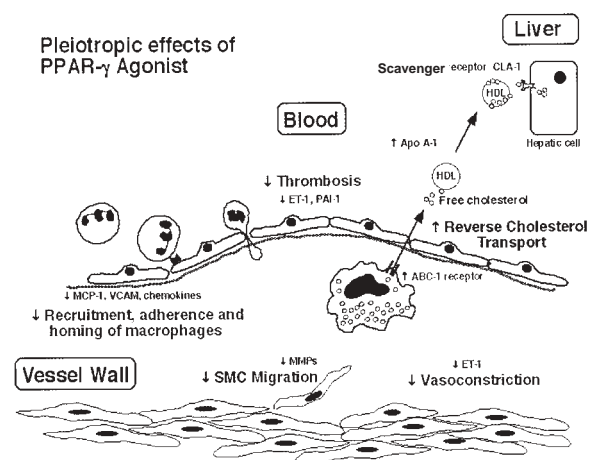


Fig. 5. Pleiotropic effects of peroxisomal proliferator-activated receptors (PPARs). PPARs are steroid hormone nuclear receptors that control a variety of cellular functions that may have the potential for inducing plaque regression and stabilization.

ABC-1 = adenosine triphosphate-binding cassette transporter-1; APO -A1 = apolipoprotein A1; CLA-1 = class B type 1 human homolog; ET-1 = endothelin-1; HDL = high-density lipoprotein; MCP-1 = monocyte chemoattractant protein-1; MMPs = matrix metalloproteinases; PAI-1 = plasminogen activator inhibitor-1; SMC = smooth muscle cell; VCAM = vascular cell adhesion molecule

macrophages (62). In early atheromas, PPAR- γ was found in foam cells and SMCs (63, 64). Several studies reported that PPAR- γ activators might reduce plaque inflammation (65, 66) by inhibiting activation of many proinflammatory genes responsible for plaque development and growth. PPAR- γ agonists also inhibit expression of adhesion molecules (67) and cytokines (66), and reduce production of matrix metalloproteinases (MMPs) (64). Evidence indicates that PPAR- γ activators can decrease thrombogenicity by reducing plasminogen activator inhibitor-1 (PAI-1) and fibrinogen concentrations, thus improving fibrinolysis (68). In addition, PPAR- γ agonists may reduce production of endothelin-1, a potent vasoconstrictor and an important atherogenic stimulus. Crucially, PPAR- γ agonists enhance reverse cholesterol transport by upregulating the genes responsible for scavenger receptor class B type I human homolog (CLA-1) (69), adenosine triphosphate-binding cassette transporter-1 (ABC-1) and apolipoprotein A1 (Apo A1), therefore facilitating efflux of free cholesterol from the plaque and its transport to the liver. These *in vitro* data suggest that PPAR- γ agonists have the potential pleiotropic effects required to induce plaque regression and stabilization.

Microembolization and Microvascular Obstruction

Plaque rupture, occurring either spontaneously or during percutaneous coronary interventions, results in the release of prothrombotic material into the coronary circulation, which may embolize the microvascular bed. This phenomenon leads to a markedly reduced myocardial function, whereas coronary blood flow remains unchanged or is even increased. The increase in coronary blood flow is attributed to vasodilation of adjacent nonembolized vessels in response to adenosine release from the microembolized myocardium. The loss of contractile function in microembolized myocardium is attributed to inflammatory processes, in particular tumor necrosis factor- α (70).

Evidence for the occurrence of distal embolization in the pathogenesis of acute coronary syndrome (ACS) is found in autopsy studies demonstrating the presence of platelet and platelet/fibrin microemboli in the cardiac tissue of patients who died suddenly or of acute myocardial infarction (MI). Prevalence in these studies was 50–80% (71). These platelet aggregates are likely to represent emboli rather than

merely platelet hyperactivity, because they are located distal to epicardial coronary arteries containing thrombus overlying a ruptured atheromatous plaque instead of being distributed more evenly in the myocardium. In addition, embolization of cholesterol and atheromatous material has been demonstrated (71). In the setting of acute MI, the presence of distal embolization is assessed clinically by looking for the presence of microvascular obstruction after reperfusion. This is known as the “no-reflow” phenomenon. Because it is demonstrated very early in the course of MI, microvascular obstruction is most likely caused by embolization rather than by edema or reperfusion injury (72).

Depression

Since established cardiovascular risk factors do not account for all patients who develop ACS events, the search for “nontraditional” risk factors has emerged (Fig. 2). Many of the immune-inflammatory factors playing a role in plaque instability have been associated with psychological factors. Depression is associated with enhanced activation of platelets and with enhanced release of β -thromboglobulin (73). Depression is also associated with elevated interleukin-6 (IL-6), which can activate platelets as well. In a recent study (74), it was demonstrated that 5-hydroxytryptamine (5-HT)-mediated platelet reactivity is significantly increased in depressed patients relative to nondepressed matched controls. However, it should be noted that adenosine diphosphate (ADP; a non 5-HT agonist) did not differentiate depressed patients from their controls, as had been previously reported by other investigators (75).

“Vulnerable Blood” and “Vulnerable Patient”

In one-third of the cases of ACS, particularly in sudden coronary death, there is no disruption of lipid-rich plaque but only superficial erosion of markedly stenotic and fibrotic plaque (76). Thrombus formation in such cases may depend on a hyperthrombogenic state triggered by systemic factors. Indeed, systemic factors, including elevated low-density lipoprotein (LDL) cholesterol, cigarette smoking, hyperglycemia, hemostasis and others, are associated with increased blood thrombogenicity (44).

Elevated LDL cholesterol levels have been found to increase blood thrombogenicity and growth of thrombi under defined rheology conditions (77, 78). Reducing LDL cholesterol lev-

els with statins was shown to decrease thrombus growth by approximately 20% (78). The question is, to what extent does such an anti-thrombotic effect, for example with statins (documented in large prospective clinical trials), contribute to the reduction of total vascular events, including death, coronary events, and stroke (79, 80)?

Smoking increases sympathetic nerve activity (81) and, therefore, catecholamine release, which may potentiate platelet activation and increase fibrinogen levels. Catecholamine-dependent effects in the circulating blood could explain not only the increase in the incidence of sudden death and acute cardiovascular events after emotional and physical stress, but also the circadian distribution of these events (82).

Diabetic patients, especially those with poorly controlled diabetes, have increased blood thrombogenicity (21). Platelets from patients with diabetes have been shown to have increased reactivity and hyperaggregability due to increases in a variety of activation-dependent adhesion proteins on their surfaces (83). Abnormal platelet function is reflected by increased platelet consumption and increased accumulation of platelets on the altered vessel wall.

Recent observations indicate that the hyperthrombogenic states associated with high LDL cholesterol, cigarette smoking, and diabetes may share a common biological pathway: activation of leukocyte-platelet interactions associated with the release of TF and thrombin activation. Specifically, more leukocyte-platelet aggregates circulate in the blood of patients with diabetes mellitus than in that of nondiabetic patients. The prothrombotic state in diabetes is also associated with an increased expression of monocyte procoagulant activity in the presence of diabetic microalbuminuria. The increased procoagulant activity in diabetes is attributed to leukocytes, which may in part activate the TF pathway (84, 85) and contribute to high blood thrombogenicity in diabetic patients (83).

Recent studies have found increased levels of circulating TF antigen in patients with cardiovascular disease (86). Circulating TF has been associated with increased blood thrombogenicity in patients with unstable angina (87) and chronic coronary artery disease. Blood levels of TF have also been shown to predict outcome in patients with unstable angina (88).

As previously described, lipid-rich atherosclerotic plaques contain TF associated with macrophages within the lesion (47) which may account, in large part, for the high thrombo-

genicity of these lesions. Tissue factor has also been identified within thrombi formed in the coronary arteries. In addition, specific inhibition of the TF pathway by TF pathway inhibitor (TFPI) significantly reduces plaque thrombogenicity (45). The TFPI is usually expressed in the adventitial layer of large arteries, and in atherosclerotic vessels TFPI is expressed by macrophages in focal areas in the plaque. Local production of TFPI may regulate procoagulant activity and thrombotic events within atherosclerotic plaques (89).

In addition to apoptotic macrophages and microparticles from atherosclerotic plaques, activated monocytes in the circulating blood seem to be a source of TF microparticles and may represent the result of activation by the previously mentioned risk factors and others, thus contributing to thrombotic events. Within the context of the possibly proinflammatory or prothrombotic effects on the circulating blood exerted by high LDL cholesterol, cigarette smoking and diabetes, there is increasing evidence that circulating monocytes and white blood cells may be involved in TF expression and thrombogenicity (86). Indeed, the predictive value for coronary events of high levels of C-reactive protein (CRP) may be a manifestation of such systemic phenomena (39).

C-reactive protein (CRP), similar to fibrinogen, is a protein of the acute-phase response and a sensitive marker of low-grade inflammation. Increased levels of CRP have been reported to predict acute coronary events (38), and CRP seems to be a useful marker in the prediction of thrombotic events. Whether CRP reflects the inflammatory component of atherosclerotic plaques or of the circulating blood, and whether it is a surrogate marker or a biologically active element in plaque development of thrombus formation at the site of the atherosclerotic vessel, are not known (90). Nevertheless, recent studies support the hypothesis that CRP is an activator of blood monocyte and vessel-wall endothelial cells (91).

New Antithrombotic Therapies

Understanding the molecular mechanisms that underlie atherothrombotic disease has been critical in developing optimal pharmacologic therapies. Given the key role of thrombosis, pharmacologic therapy has sought to provide potent inhibition of both platelet aggregation and coagulation cascade. Traditionally, antiplatelet therapy with aspirin (92) and clopido-

grel, and anticoagulation therapy with unfractionated heparin (UFH) (93), low molecular weight heparin (LMWH) and warfarin, have been the cornerstone of management of patients with atherothrombosis. Despite the beneficial effects of these agents, they have limitations, and novel antithrombotic agents have been developed to address these limitations. Given the critical role of TF in atherothrombosis, different therapeutic approaches directed toward inhibiting the TF pathway have been investigated (Fig. 6).

Three groups of agents inhibiting TF pathway are being developed. The first group consists of specific TF inhibitors, the second contains factor Xa inhibitors, and the third includes direct thrombin inhibitors (DTI). Within the first group, recombinant TFPI is available as a recombinant drug. In order to avoid some bleeding complications associated with systemic administration of recombinant TFPI, transfer of human tissue factor pathway inhibitor gene has recently been accomplished by using an adenovirus as a vector (94). Another novel therapeutic approach is the inhibition of TF gene transcription. The nuclear factor NF- κ B pathway is a key transcriptional mechanism in induction of the TF gene. Dithiocarbamates, specific inhibitors of this pathway, reduced TF expression in preclinical studies (95). The blockade of TF-induced thrombin generation was studied by using inactivated factor VIIa, a

competitive inhibitor of TF-dependent factor X activation. In both animal models and studies in humans, thrombus formation was prevented by infusing inactivated factor VIIa (96). Studies in animals and more recently in humans have demonstrated the efficacy of TF monoclonal antibodies in reducing thrombosis (97). A peptide with a mode of action comparable to TFPI is a nematode anticoagulant protein c2 (NAPc2). A recombinant form of NAPc2 was tested in an animal model and showed promising results (98).

Selective inhibitors of factor Xa can be classified as direct, those which bind directly to factor Xa, or indirect, such as fondaparinux, which require antithrombin III for their action.

The DTIs include hirudin, bivalirudin, and a second generation of agents, called active site inhibitors. Melagatran, together with its oral prodrug (ximelagatran) is the most extensively investigated of this last group of drugs. Recent studies on TF pathway inhibitors are summarized in the Table (45, 78, 94–120).

Conclusion

In this review we wanted to emphasize the following concepts:

1. Atherothrombosis should be used to define atherosclerosis and its thrombotic complications. This term refers not only to the classical coronary syndromes, but also to cerebrovascular and peripheral artery diseases.
2. The concept of “vulnerable blood” explains those clinical situations characterized by thrombus formation in the presence or absence of plaque disruption.
3. Inflammation and endothelial dysfunction are of paramount importance as determinants of “vulnerability” of flowing blood.
4. High levels of circulating tissue factor activity have been associated with those subpopulations of CAD patients particularly prone to thrombotic complications (diabetics, smokers, dyslipidemic).
5. Vascular cells undergoing apoptosis seem to be a source for observed increase of circulating TF activity in these subpopulations.
6. Inhibition of tissue factor metabolic pathway is being introduced as a novel and effective antithrombotic target.

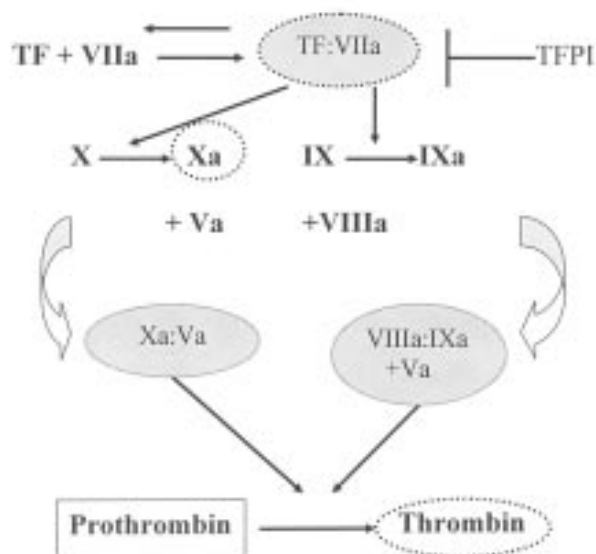


Fig. 6. Tissue factor pathway and potential therapeutic targets. As described in the picture, the binary complex TF:VIIa is of critical importance in initiating the coagulation cascade, and therefore it represents a very attractive pharmacologic target (within dashed circle). TF = tissue factor; TFPI = tissue factor pathway inhibitor

TABLE
Summary of Recent Studies on Tissue Factor Pathway Inhibitors

Therapy	Mechanism of Action	Model	References
Tissue Factor Inhibitors			
Tissue factor pathway inhibitor (TFPI)	Direct binding to factor Xa Inhibition of TF:VIIa complex	Animal Animal	Oltrona L et al. (99) Han X et al. (100) Bajaj MS and Bajaj SP (101)
Gene transfer of TFPI	Local expression of TFPI	<i>In vitro</i> Animal	Badimon JJ et al. (45) Atsuchi N et al. (102) Golino P et al. (94)
Monoclonal antibodies	Inhibition of TF activity	Animal	Ragni M et al. (103) Annex BH et al. (97)
Dithiocarbamates	Inhibition of TF gene transcription	<i>In vitro</i> Animal	Rauch U et al. (78) Drollinger AG et al. (104) Bohrer H et al. (95)
Active site-inactivated factor VIIa (Approved for clinical use)	Competitive inhibition of TF-dependent activation of factor Xa	Animal	Banner DW et al. (96)
Nematode anticoagulant protein c2 (In clinical development)	Inhibition of TF:VIIa complex	<i>In vitro</i> Animal Human	Jang Y et al. (105) Rote WE et al. (98) Lee A et al. (106)
Factor Xa Inhibitors			
Tick anticoagulant peptide	Direct inhibition of factor Xa	Animal <i>In vitro</i>	Beimond BJ et al. (107) Orvim U et al. (108)
Antistatin DX9065 (In clinical development)	Direct inhibition of factor Xa	Animal	Ragosta M et al. (109)
Fondaparinux (Approved for clinical use)	Indirect inhibition of factor Xa	Human	Shimbo D et al. (110) Turpie AG et al. (111)
Thrombin Inhibitors			
Hirudin (Approved for clinical use)	Direct thrombin inhibitor	Human	OASIS-1 & 2 (112)
Bivalirudin (Approved for clinical use)	Direct thrombin inhibitor	Human	CACHET (113)
Argatroban (Approved for clinical use)	Active site thrombin inhibitor	Human	ARGAMI (114)
Melagatran (In clinical development)	Active site thrombin inhibitor	Human	METHRO I, II, III (115–117)
Ximelagatran (In clinical development)	Active site thrombin inhibitor	Human Human Animal	METHRO I, II, III (115–117) SPORTIF I, II, III (118, 119) Carlsson S et al. (120)

Despite the advances in our understanding of the pathophysiology of this disease and the improvement of the therapeutic armamentarium, atherothrombosis continues to be the leading cause of mortality in most of the Western world.

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