CHAPTER 3
Vascular biology of acute coronary syndromes

Lina Badimon

Introduction

Thrombus formation in a coronary artery with obstruction of coronary blood flow and reduction in oxygen supply to the myocardium produces the acute coronary syndromes (ACSs). These thrombotic episodes largely occur in response to atherosclerotic lesions that have progressed to a high risk-inflammatory/prothrombotic stage. Although apparently distinct, the atherosclerotic and thrombotic processes appear to be closely interrelated as the causal presentation of ACS through a complex multifactorial process named atherothrombosis. ACS represents a spectrum of ischemic myocardial events that share similar pathophysiology and include unstable angina, non-Q-wave myocardial infarction, Q-wave myocardial infarction, and sudden death.

Atherosclerosis is a chronic systemic disease involving the intima of large- and medium-sized arteries including the aorta, carotids, coronaries, and peripheral arteries, which is characterized by intimal thickening due to cellular- and lipid-accumulation [1]. Endothelial dysfunction and inflammation are the major facilitators of atherosclerotic disease progression. Lipid accumulation results from an imbalance between mechanisms responsible for lipid influx and efflux. Secondary changes may occur in the underlying media and adventitia, particularly in advanced disease stages. Indeed, fibroblasts from the adventitia have been shown to have an important partnership with the medial smooth muscle cells (SMCs), resulting in neointima formation and compensatory vascular enlargement (remodeling). The early atherosclerotic lesions might progress without compromising the lumen due to this remodeling [2]. When fatty streaks progress to fibroatheroma, they develop a cap of SMCs and collagen, and when this plaque is disrupted, the subsequent thrombus formation is the first step of ACS and strokes. Importantly, the culprit lesions leading to ACS are usually mildly stenotic and therefore are poorly detected by angiography [3]. The composition of the plaque, rather than the stenosis, appears to be the main determinant of risk of plaque rupture and following thrombogenicity. High-risk rupture-prone lesions usually have a large lipid core, a thin fibrous cap, high density of inflammatory cells (particularly at

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the shoulders of the plaque where disruptions most often occur) and a high tissue factor (TF) content [4,5]. Inflammatory processes also contribute markedly to atherosclerosis and its acute thrombotic complications, as is shown by the fact that many inflammatory mediators can augment TF gene expression by vascular cells, thus triggering the coagulation cascade [6]. Due to the baffling heterogeneity in the composition of atherothrombotic plaques even within the same individual, a reliable, noninvasive imaging tool able to detect early atherosclerotic disease and characterize lesion composition would be clinically relevant. Indeed, it would improve our understanding of the pathophysiological mechanisms of atherothrombosis and help in patient risk stratification [7]. The variety of lesion types is shown by the fact that two-thirds of ACSs take place after the disruption of a high-risk atherothrombotic plaque with superimposed thrombus formation, but, on the other hand, in one-third of the cases there is no plaque rupture but a superficial erosion of a markedly stenotic and fibrotic lesion [8]. In the latter, thrombosis may be triggered by a hyperthrombogenicity due to systemic factors [9].

Growing thrombi on atherosclerotic vessels may locally occlude the lumen, or embolize and be washed away by the blood flow to occlude distal vessels. However, thrombi may be physiologically and spontaneously lysed by mechanisms that block thrombus propagation. Thrombus size, location, and composition are regulated by (1) hemodynamic forces (mechanical effects); (2) thrombogenicity of exposed substrate (local molecular effects), which are strongly related to the degree of plaque disruption or substrate exposure, relative concentration of fluid phase, and cellular blood components (local cellular effects); and the (3) efficiency of the physiologic mechanisms to control the system, mainly fibrinolysis [10].

Cellular and molecular mechanisms in atherogenesis

Role of lipids and lipoproteins

Cholesterol is transported into the vessel wall as a component of the lipoproteins. Low-density lipoprotein (LDL) is considered the most atherogenic lipoproteins since they accumulate in the intima and carry large amounts of plasmatic cholesterol (up to 70%).

High levels of circulating LDL are predictive markers for cardiovascular disease. It appears that discrete LDL subclasses carry different levels of atherogenicity. The "atherogenic lipoprotein phenotype" describes a combination of moderate hypertriglyceridemia, low high-density lipoprotein (HDL) – cholesterol, and a predominance of small, dense LDL particles. This dyslipemia is prevalent in patients with the metabolic syndrome, in those with Type II diabetes, and in postmenopausal women.

Dietary fats and oils differ in the chain lengths of their constituent fatty acids and the number and geometry of their double bonds. These differences markedly affect concentrations of lipids in plasma and differences in

the amount and type of fat from the diet can induce differences of 30–40% in serum LDL concentrations. When saturated fatty acids are replaced by unsaturated fats, total plasma cholesterol is lowered. A review of metabolic studies, prospective cohort studies, and clinical trials indicates that there are multiple mechanisms by which diet potentially influences risk of congenital heart disease (CHD), and there are dietary strategies effective in preventing CHD. As such, the substitution of nonhydrogenated unsaturated fats for saturated and trans-fats, the increase in the consumption of omega-3 fatty acids from fish, fish oil supplements, or plant sources, and the consumption of a diet high in fruits, vegetables, nuts, and whole grains and low in refined grain products are good strategies to prevent CHD [11].

Extracellular accumulation of lipids in the arterial intima occurs very early in response to increased plasma lipoproteins levels. Proteoglycan (PG) and protein-bound lipoprotein particles, perhaps, in a microenvironment shielded from plasma antioxidants, can undergo modifications. Such modifications include oxidation, aggregation, enzymatic, and nonenzymatic modifications of LDL. Modified forms of LDL are associated with increased atherogenicity because the physicochemical properties of the lipoprotein become altered. This may change the biological properties of the LDL and also increase the susceptibility of the LDL to other types of modifications.

Endothelial dysfunction

Hypercholesterolemia induces an increase of leukocyte recruitment by an increased expression of adhesion molecules per se and those induced by cytokines, a decrease in endothelium-dependent vasodilatation, and alterations in the thrombosis/fibrinolysis balance. Recently, our group has demonstrated that hypercholesterolemia can induce endothelial dysfunction by altering the expression of genes that are regulated through the downregulation of sterol regulatory element binding proteins (SREBPs) in endothelial cells [12].

Synthesis and degradation of extracellular matrix

Modified LDL can modulate the synthesis of PGs in different cell types. The increase of PG synthesis induced by LDL might have important consequences for the intimal LDL retention. Additionally, the exposure of endothelial cells to apoE-containing HDL has also shown to stimulate the production of heparan sulfate proteoglycans (HS-PGs) that have increased sulfation [13].

Lipoproteins also modulate the expression of matrix metalloproteinases (MMPs), enzymes that are able to digest various connective tissue components. An increase in the expression and activity of metalloproteinases is associated with the disruption of the fibrous cap of lesions and plaque rupture. Additionally, the breakdown products of the extracellular matrix (ECM) may be biologically active and might increase processes that are fundamental for the pathogenesis of the atherosclerosis.

Foam cell formation

Modified lipoproteins are taken up through mechanisms not regulated by cholesterol, leading to high intracellular cholesteryl ester accumulation and foam cell formation. The accumulation of lipid-laden foam cells is one of the earliest steps in the progression of the atherosclerotic plaque. In macrophages, the scavenger receptors (SRs) are mainly responsible for modified LDL uptake. We have recently described a main role for low density lipoprotein receptor-related protein (LRP) in the uptake of agLDL by vascular smooth muscle cells (VSMCs) [14]. SR and the LRP have been detected in human atherosclerotic lesions and could, therefore, play an important role in foam cell formation in the arterial intima. Several modified lipoproteins have demonstrated to upregulate their own receptors, for example, oxLDL increase the expression of several SRs, such as CD36, SR-A, and LOX-1, in different cell types. In addition, agLDL upregulate the expression of their receptor LRP in VSMCs [15]. Most of the modified lipoproteins could lead to a progressive cholesteryl ester accumulation not only by being taken up through non-downregulated receptors, but also by upregulating their own receptors.

Lipoproteins and the metabolic syndrome

The metabolic syndrome has recently been defined by the National Institutes of Health (2001) [16] as a cluster of disorders including abdominal obesity, insulin resistance, diabetes, endothelial dysfunction, blood pressure, and impaired fibrinolysis. The risk factors that constitute the metabolic syndrome consist of atherogenic dyslipemia, elevated blood pressure, elevated plasma glucose, and a prothrombotic state [17]. The metabolic syndrome is closely linked to the metabolic derangement called insulin resistance. This condition is characterized by a generalized defect in the insulin-signaling pathway [18]. Because insulin induces a myriad of metabolic responses, a defect in insulin-signaling results in several metabolic changes. The presence of insulin resistance predisposes to the development of Type II diabetes. There are four major causes of insulin resistance: genetics, obesity, lack of exercise, and diet composition. Insulin resistance and metabolic syndrome are related to the atherogenic dyslipemia also called "atherogenic lipoprotein phenotype" characterized by increased triglyceride-rich lipoproteins, increased small LDL particles, and reduced levels of HDL [19]. These changes in lipoprotein and fatty acid profile observed in insulin resistance may influence different proatherosclerotic mechanisms such as membrane lipid composition, metabolism or signal-transduction pathways [20].

Role of inflammation

Different constituents of the modified lipoproteins trigger the production of mediators of innate immunity. There are also nonlipid mediators involved in

inflammation, such as homocystein, angiotensin II, and microbial products that can induce the synthesis of cytokines in atheroma-associated cells.

Under normal circumstances, the endothelial monolayer in contact with flowing blood is inert to the adhesion of leukocytes. The situation changes in dysfunctional endothelium. One of the endothelial–leukocyte adhesion molecules mainly involved in the early adhesion of mononuclear leukocytes to arterial endothelium is VCAM-1, which binds particularly those classes of leukocytes found in nascent atheroma: the monocyte and the T-lymphocyte. In addition to VCAM-1, P- and E-selectin, and ICAM-1 also seem to contribute to leukocyte recruitment. After leukocyte adhesion to the endothelium, the cells enter into the intima by diapedesis, a process that is facilitated by different chemokines, such as MCP-1, IL-8, and a trio of CXC chemokines induced by interferon-γ. Once in the intima, monocytes acquire characteristics of macrophage by expressing certain scavenger receptors, such as scavenger receptor A (SRAI and II) and CD36, that internalize modified lipoproteins leading to foam cell formation (as previously described).

One of the most important clinical markers of inflammation is C-reactive protein (CRP), which has been shown in multiple epidemiological studies to predict incidence of myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death. In terms of clinical application, some data indicate that CRP seems to be a strong predictor of cardiovascular events and it adds prognostic information at all levels of calculated Framingham risk and at all levels of metabolic syndrome [21]. The feature that distinguishes CRP from LDL cholesterol is the fact that inflammation (but not elevated cholesterol LDL) plays a major role in almost all processes associated with the metabolic syndrome.

Role of the different components of the vascular wall

Extracellular matrix

The ECM of the arterial intima is a relatively large compartment made of collagen, elastin, complex PGs, and hyaluronate and multidomain proteins such as fibronectin, laminin, and tenascin [13]. ECM occupies 60% of the arterial intima and regulates numerous cellular functions. The main PGs structuring the ECM are chondroitin sulfate proteoglycans CS-PGs, such as versican or biglycan, which have the longest negatively charged glycosaminoglycan (GAG) chains and are mainly synthesized by VSMC. HS-PGs, such as perlecan, are constituents of the basement membrane and are mainly synthesized by endothelial cells and VSMCs. In addition, other HS-PGs, such as syndecans and glypicans, are found in the cell membranes of the vascular cells. While CS-PGs play a major role for LDL retention in the arterial intima, cell-surface HS-PGs are dynamic molecules that mediate ligand catabolism [22]. Collagens play a central role not only in maintaining the integrity and stability of the wall, but also in many cellular functions.

Cellular components

Endothelium

Normally, vascular endothelium forms a multifunctional interface between circulating blood and the various tissues and organs in the body. It constitutes a selectively permeable barrier for macromolecules, as well as a nonthrombogenic surface that actively maintains the fluidity of blood. Endothelial dysfunction, as well as a breach of the endothelial integrity, triggers a series of biochemical and molecular reactions aimed at arresting blood flow and vessel wall repair. One of its main traits is the reduction of the availability of vasodilators, especially nitric oxide (NO), and an increase in endothelium-derived contracting factors leading to vasoconstriction, such as angiotensin II (AGII).

These vasoactive substances mediate vascular tone, structure, and function influencing VSMC growth, apoptosis, platelet aggregation, monocyte and leukocyte adhesion, and thrombosis. The homeostasis of vasoactive substances is disrupted by endothelial dysfunction, leading to changes in vascular structure and function. Hypertension and other risk factors for cardiovascular disease are associated with endothelial dysfunction and vascular remodeling. Elevated AGII activity, which is strongly correlated with hypertension, is a major trigger of endothelial dysfunction in hypertensive patients. AGII stimulates NADPH/NADH oxidase in endothelium, VSMC, and adventitia of blood vessels to generate reactive oxygen species, leading to endothelial dysfunction, cell growth, and inflammation [23]. These changes result in upregulation of endothelin-1, adhesion molecules, NF-kb, and other inflammatory mediators, as well as increased breakdown of nitric oxide and uncoupling of nitric oxide synthase. The balance of vasoconstriction and vasodilatation is thus disrupted, leading to vascular remodeling and injury. Physiopathological stimuli especially relevant to atherogenesis include cytokines and bacterial endotoxins, infection by viruses, advanced glycosylation end products that are generated in diabetes and with aging, hyperhomocysteinemia, and hypercholesterolemia, and oxidized LDL. In addition to these humoral stimuli, it is also clear that biochemical forces generated by flowing blood can also influence endothelial cell structure and function, modulating the expression of relevant genes. Hemodynamic effects on endothelium are supported by the long-standing observation that the earliest lesions develop in a nonrandom pattern, the geometry of which correlates with branch points and other regions of altered blood flow. A variety of changes in the metabolic and synthetic activities of endothelial cells in response to defined biomechanical forces have been reported. These include the production of arachidonate metabolites, growth factors, coagulation and fibrinolytic factors, ECM components, and vasoactive mediators. Certain of the most acute shear-induced changes appear to involve regulation at the level of rate-limiting enzymes and/or substrate availability. However, especially in the case of delayed responses, in which *de novo* synthesis occurs, upregulation of gene expression appears to occur as a direct consequence of exposure to fluid mechanical forces. There are genes such as *PDGF-B*, *MCP-1*,

VCAM-1, and *endothelin-1* that have a "shear stress response element" in their promoter.

Monocyte/macrophages

The state and function of the macrophages in the atherosclerotic lesions may be critical for the development of atherosclerosis. Macrophages play an important role in innate immune responses, cellular adhesion, phagocytosis of apoptotic cells, and lipid uptake. Most of these macrophage functions are mediated by SRs. Since the cloning of the first two macrophage SR (now called SR-A Type I and Type II), the broad SR family has grown considerably. On the basis of functional studies and expression in the arterial intima, only some of the SRs are good candidates to contribute to atherosclerotic foam cell formation. Besides its role in lipid accumulation, macrophages may also contribute to atherosclerosis through secretory inflammatory products. Activated lymphocytes and macrophages with a wide expression of Class II histocompatibility antigen have been found at every stage of atherosclerotic lesions indicating that macrophages may participate in local immune responses.

Vascular smooth muscle cells

Vascular smooth muscle cells represent an average of 50% of the cellular component in advanced atherosclerotic plaque and may reach 90–95% in early lesions. In response to multiple stimuli, VSMC from the arterial tunica media are activated and migrate to the intima where they proliferate. These seem to be early steps at the onset of the atherosclerotic process. The proliferation and migration of VSMC from the media to the intima is one of the key events in early atherosclerosis. The understanding of the molecular mechanisms involved in VSMC activation and differentiation requires an accurate mapping of the cascade of transcription factors induced by atherogenic stimuli. Recently, different nuclear receptors including PPARs, retinoid receptors, retinoid X receptors (RXRs), retinoic acid receptors (RARs), and retinoid-related orphan receptors (RORs) have been identified in VSMC activation/proliferation, and consequently have been involved in atherogenesis [24,25]. Recently our group has identified neuron-derived organ receptor-1 (*NOR-1*) as a new early response gene in VSMCs [26]. *NOR-1* is strongly induced by growth factors and thrombin, and is overexpressed in atherosclerotic lesions from patients with coronary artery disease (CAD). It is transiently induced by PTCA in porcine coronary arteries. It is also induced by high cholesterol levels [27]. These results suggest that *NOR-1* may play a role in the molecular mechanisms underlying both spontaneous and accelerated atherosclerosis.

Vascular smooth muscle cells also contribute to the lesion by synthesizing ECM. In fact, proliferative VSMCs have a high capacity to synthesize sulfated-PGs, and it is well established that PGs in the arterial wall are involved in the focal deposition of cholesterol-rich particles in the early phases of atherogenesis.

Finally, VSMCs also have a great importance in foam cell formation. We demonstrated that agLDL can cause high intracellular cholesteryl ester accumulation in VSMCs [14,15,28]. We showed, for the first time, that in VSMCs, LRP mediates the binding and internalization of agLDL and that in absence of LRP function, VSMCs are unable to accumulate cholesterol. Additionally, this receptor is expressed in atherosclerotic plaques [29] and it is upregulated by agLDL uptake, leading to intracellular lipid accumulation.

Cellular and molecular mechanisms in thrombus formation

The endothelium is an active organ system playing a crucial role in maintaining vascular homeostasis via regulation of hemostatic, inflammatory, and reparative responses to local injury, and it has many antithrombotic and fibrinolytic properties. Vasoconstriction and platelet adhesion at the place of vascular injury cooperate to form a hemostatic aggregate, as the first step in vessel wall repair and the prevention of excessive blood loss. A few scattered platelets may interact with subtly injured, dysfunctional endothelium, and contribute by the release of growth factors to intimal hyperplasia. In contrast, from a monolayer to a few layers of platelets may deposit on the lesion with mild injury that may or may not evolve to a mural thrombus. The release of platelet growth factors may contribute significantly to an accelerated intimal hyperplasia, as it occurs in the coronary vein grafts within the first postoperative year. In severe injury, with exposure of components of deeper layers of the vessel, as in spontaneous plaque rupture or in angioplasty, marked platelet aggregation with mural thrombus formation follows. Vascular injury of this magnitude also stimulates thrombin formation through both the intrinsic (surface-activated) and extrinsic (tissue-factor dependent) coagulation pathways, in which the platelet membrane facilitates interactions between clotting factors.

Platelets

After plaque rupture, some of the atherosclerotic plaque components exhibit a potent activating effect on platelets and coagulation [30–32]. Exposed matrix from the vessel wall and thrombin generated by the activation of the coagulation cascade as well as epinephrine and ADP are powerful platelet agonists. Each agonist stimulates the discharge of calcium and promotes the subsequent release of its granule content. Although platelets are classically relegated to this passive role as a responder to thrombotic stimuli, they are also increasingly evincing importance as a source of inflammatory mediators. For example, they can both produce and respond to chemoattractant cytokines [33], or express CD154 (CD40 ligand), the molecule which regulates TF gene expression in the macrophage and SMCs [34]. Platelet-related ADP and 5HT stimulate adjacent platelets, further enhancing the process of

platelet aggregation. Arachidonate, which is released from the platelet membrane by the stimulatory effect of collagen, thrombin, ADP, and 5HT, promotes the synthesis of thromboxane A_2 by the sequential effects of cyclooxygenase and thromboxane synthetase. Thromboxane A_2 not only promotes further platelet aggregation, but is also a potent vasoconstrictor.

The initial recognition of damaged vessel wall by platelets involves (1) adhesion, activation, and adherence to recognition sites on the thromboactive substrate [ECM proteins e.g. von Willebrand factor (vWF), collagen, fibronectin, vitronectin, laminin]; (2) spreading of the platelet on the surface; and (3) aggregation of platelets with each other to form a platelet plug or white thrombus. The efficiency of the platelet recruitment will depend on the underlying substrate and the local geometry (local factors). A final step of recruitment of other blood cells also occurs; erythrocytes, neutrophils, and occasionally monocytes are found on evolving mixed thrombus.

Platelet function depends on adhesive interactions and most of the glycoproteins on the platelet membrane surface are receptors for adhesive proteins. Many of these receptors have been identified, cloned, sequenced, and classified within large gene families that mediate a variety of cellular interactions [35]. The most abundant is the integrin family, which includes GPIIIbIIa, GPIaIIa, GPIcIIa, the fibronectin receptor, and the vitronectin receptor, in decreasing order of magnitude. Another gene family present in the platelet membrane glycocalyx is the leucine-rich glycoprotein family represented by the GPIbIX complex, receptor for vWF on unstimulated platelets that mediates adhesion to subendothelium and GPV. Other gene families include the selectins (such as GMP-140) and the immunoglobulin domain protein (HLA Class I antigen and platelet/endothelial cell adhesion molecule-1, PECAM-1). Unrelated to any other gene family is the GPIV (IIIa).

Another recently discovered receptor on platelets is P-selectin. P-selectin is a transmembrane protein present in the alpha granules of platelets, from where it quickly moves to the platelet surface after activation. It interacts with the P-selectin glycoprotein ligand-1 on leukocytes, forming aggregates and upregulating TF formation. It also enforces platelet aggregates through interaction with platelet sulfatides. This might explain why P-selectin expression in platelets has been linked to arterial thrombosis and coronary artery disease [36]. P-selectin is also present on activated endothelial cells, where it helps in the recruitment of leukocytes [37]. Indeed, selectins are specialized in lymphocyte homing and involved in inflammation processes.

Thrombin plays an important role in the pathogenesis of arterial thrombosis. It is one of the most potent known agonists for platelet activation and recruitment. The thrombin receptor has 425 amino acids with 7 transmembrane domains and a large NH_2-terminal extracellular extension that is cleaved by thrombin to produce a "tethered" ligand that activates the receptor to initiate signal transduction [38]. Thrombin is a critical enzyme in early thrombus formation, cleaving fibrinopeptides A and B from fibrinogen to yield insoluble fibrin, which effectively anchors the evolving thrombus. Both

free and fibrin-bound fibrin thrombin are able to convert fibrinogen to fibrin, allowing propagation of thrombus at the site of injury.

Therefore, platelet activation triggers intracellular signaling and expression of platelet membrane receptors for adhesion and initiation of cell contractile processes that induce shape change and secretion of the granular contents. The expression of the integrin IIb/IIa (αIIbβ_3) receptors for adhesive glycoprotein ligands (mainly fibrinogen and vWF) in the circulation initiates platelet-to-platelet interaction. The process becomes perpetuated by the arrival of platelets brought by the circulation. Most of the glycoproteins in the platelet membrane surface are receptors for adhesive proteins or mediate cellular interactions. vWF has been shown to bind to platelet membrane glycoproteins in both adhesion (platelet–substrate interaction) and aggregation (platelet–platelet interaction), leading to thrombus formation in perfusion studies conducted at high shear rates [35]. Ligand binding to the different membrane receptors triggers platelet activation with different relative potencies. A lot of interest has been recently generated on the platelet ADP-receptors ($P2Y_{AC}$, P2y1R, $P2X_{1R}$) because of available pharmacological inhibitors.

Activation of the coagulation system

During plaque rupture, in addition to platelet deposition in the injured area, the clotting mechanism is activated by the exposure of the plaque contents. The activation of the coagulation leads to the generation of thrombin, which is a powerful platelet agonist in addition to an enzyme that catalyzes the formation and polymerization of fibrin. Fibrin is essential in the stabilization of the platelet thrombus and its withstanding to removal forces by flow, shear, and high intravascular pressure. The efficacy of fibrinolytic agents pointedly demonstrates the importance of fibrin in thrombosis associated with myocardial infarction.

The blood coagulation system involves a sequence of reactions integrating zymogens – proteins susceptible to be activated to enzymes (via limited proteolysis) and cofactors (nonproteolytic enzyme activators) in three groups: (1) the contact activation (generation of factor XIa via the Hageman factor); (2) the conversion of factor X to factor Xa in a complex reaction requiring the participation of factors IX and VIII; and (3) the conversion of prothrombin to thrombin and fibrin formation [35,39].

Glycosaminoglycans and sulfatides have been suggested to be the triggering surfaces for *in vivo* initiation of contact activation; however, the physiologic role of coagulation contact activation is unclear, since the absence of Hageman factor, prekallikrein, or high-molecular-weight kininogen does not induce a clinically apparent pathology. On the contrary, factor XI deficiency is associated with abnormal bleeding. Activated factor XI induces the activation of factor IX in the presence of Ca^{2+}. Factor IXa forms a catalytic complex with factor VIII on a membrane surface and efficiently activates factor X in the presence of Ca^{2+}. Factor IX is a vitamin K-dependent enzyme, as are factor VII, factor X, prothrombin, and protein C.

The TF pathway, previously known as extrinsic coagulation pathway, through TF factor VIIa complex in the presence of Ca^{2+} induces the formation of Xa. A second TF-dependent reaction catalyzes the transformation of IX into IXa. TF is an integral membrane protein that serves to initiate the activation of factors IX and X and localize the reaction to cells on which TF is expressed. Other cofactors include factor VIIIa, which binds to platelets and forms the binding site for IXa, thereby forming the machinery for the activation of X, and factor Va, which binds to platelets and provides a binding site for Xa. The human genes for these cofactors have been cloned and sequenced. In physiologic conditions, no cells in contact with blood contain active TF, although cells such as monocytes and polymorphonuclear leukocytes can be induced to synthesize and express TF [39].

Activated Xa converts prothrombin into thrombin. The complex, which catalyzes the formation of thrombin, consists of factors Xa and Va in a 1 : 1 complex. The activation results in the cleavage of fragment 1.2 and formation of thrombin from fragment 2. The interaction of the four components of the "prothrombinase complex" (Xa, Va, phospholipid, and Ca^{2+}) yields a more efficient reaction [40].

Activated platelets provide procoagulant surface for the assembly and expression of both intrinsic Xase and prothrombinase enzymatic complexes. These complexes, respectively, catalyze the activation of factor X to factor Xa and prothrombin to thrombin. The expression of activity is associated with the binding of both the proteases, factor IXa and factor Xa, and the cofactors, VIIIa and Va, to procoagulant surfaces. The binding of IXa and Xa is promoted by VIIIa and Va, respectively, such that Va and VIIIa is likely to provide the equivalent of receptors for the proteolytic enzymes. The surface of the platelet expresses the procoagulant phospholipids that bind coagulation factors and contribute to the procoagulant activity of the cell [40].

Thrombin acts on multiple substrates, including fibrinogen, factor XIII, factors V and VIII, and protein C, in addition to its effects on platelets. It plays a central role in hemostasis and thrombosis. The catalytic transformation of fibrinogen into fibrin is essential in the formation of the hemostatic plug and in the formation of arterial thrombi. It binds to the fibrinogen central domain and cleaves fibrinopeptides A and B, resulting in fibrin monomer and polymer formation. The fibrin mesh holds the platelets together and contributes to the attachment of the thrombus to the vessel wall. Thrombin makes sure the coagulation cascade is activated and stimulates platelet aggregation, thus becoming pivotal to the stability of the mural thrombus, which forms over the plaque and releases growth factors and platelet vasoconstrictors, favoring the onset of ischemic events.

Spontaneous anticoagulation and fibrinolysis

The control of the coagulation reactions occurs by diverse mechanisms, such as hemodilution and flow effects, proteolytic feedback by thrombin, inhibition

by plasma proteins (such as antithrombin III [ATIII]) and endothelial cell-localized activation of an inhibitory enzyme (protein C), and fibrinolysis. Although ATIII readily inactivates thrombin in solution, its catalytic site is inaccessible while bound to fibrin, and it may still cleave fibrinopeptides even in the presence of heparin. Thrombin has a specific receptor in endothelial cell surfaces, thrombomodulin, that triggers a physiologic anticoagulative system. The thrombin–thrombomodulin complex serves as a receptor for the vitamin K-dependent protein C which is activated and released from the endothelial cell surface. Activated protein C, in the presence of protein S, inactivates factors Va and VIIIa and limits thrombin effects. Thrombin generated at the site of injury binds to thrombomodulin, an endothelial surface membrane protein, initiating activation of protein C, which in turn (in the presence of protein S) inactivates factors Va and VIIIa. Loss of Va decreases the role of thrombin formation to negligible levels [40]. Thrombin stimulates successive release of both tissue plasminogen activator (tPA) and plasminogen-activator inhibitor type 1 from endothelial cells, thus initiating endogenous lysis through plasmin generation from plasminogen by tPA with subsequent modulation through plasminogen-activator inhibitor type 1. Thrombin therefore plays a pivotal role in maintaining the complex balance of initial prothrombotic reparative events and subsequent endogenous anticoagulant and fibrinolytic pathways. Endogenous fibrinolysis (repair mechanism) involves catalytic activation of zymogens, positive and negative feedback control, and inhibitor blockade.

Blood clotting is blocked at the level of the prothrombinase complex by the physiologic anticoagulant-activated protein C and oral anticoagulants. Oral anticoagulants prevent posttranslational synthesis of γ-carboxyglutamic acid groups on the vitamin K-dependent clotting factors, preventing binding of prothrombin and Xa to the membrane surface. Activated protein C cleaves factor Va to render it functionally inactive. Endothelial loss, contributes to the high thrombogenicity of atherosclerotic plaques. Overall, it is likely that when injury to the vessel wall is mild, the thrombogenic stimulus is relatively limited, and the resulting thrombotic occlusion is transient, as occurs in unstable angina. On the other hand, deep vessel injury secondary to plaque rupture or ulceration results in exposure of collagen, TF, and other elements of the vessel matrix, leading to relatively persistent thrombotic occlusion and myocardial infarction [1].

It is likely that the nature of the substrate exposed after spontaneous or angioplasty-induced plaque rupture determines whether an unstable plaque proceeds rapidly to an occlusive thrombus or persists as nonocclusive mural thrombus. The analysis of the relative contribution of different components of human atherosclerotic plaques (fatty streaks, sclerotic plaques, fibrolipid plaques, atheromatous plaques, hyperplasic cellular plaque, and normal intima) to acute thrombus formation showed that the atheromatous core was up to sixfold more active than the other substrates, in triggering thrombosis [4]. The atheromatous core, together with the residual mural thrombus

[41], remained the most thrombogenic substrate when the substrates were normalized by the degree of irregularity as defined by the roughness index. Therefore ruptured plaques with a large atheromatous core are at high risk to lead to ACS, and this core shows the most intense TF staining compared with other components [4,5]. As proof of concept, we showed that local tissue blockade of TF, by treatment with TFPI, significantly reduces thrombosis [42]. Recently, the use of active site inhibited recombinant FVIIa (FF-rFVIIa) has shown to significantly reduce thrombus growth on damaged vessels without TF, indicating the efficacy of blocking blood-borne TF [43].

Monocyte/macrophage are key to the development of vulnerable plaques [44]. The vulnerable plaques (AHA Type IV and Va), commonly composed of an abundant lipid core separated from the lumen by a thin fibrotic cap, are particularly soft and prone to disruption [45]. A high density of activated inflammatory cells has been detected in the disrupted areas of atherectomy specimens from patients with acute coronary syndromes [46]. These cells are capable of degrading ECM by secreting proteolytic enzymes, such as MMPs [47], among which some can further promote thrombogenicity through the activation of platelet aggregation, as MMP-2 does [48]. In addition, T-cells isolated from rupture prone sites can stimulate macrophages to produce metalloproteinases and may predispose lesions disruption by weakening their fibrous cap. Recently, LDLs have shown to downregulate the expression of lysil-oxidase (LOX) in vascular wall cells [49]. LOX is an enzyme that contributes to the maturation of the elastin and collagen fibrils of the ECM. Its decrease is associated to an increased permeability of the vascular wall and hence may contribute to plaque destabilization. Cell apoptosis and microparticles with procoagulant activity and postulated apoptotic origin have also been linked to inflammation and thrombosis [50,51]. Finally, it merits mentioning that atherosclerotic plaques often demonstrate increased adventitial and intimal neovascularization, especially at the lesions responsible for ACSs [52,53]. These new vessels may provide a way to recruit inflammatory cells into the plaque, and due to their thin walls easily rupture and hemorrhage, with the consequent secondary plaque expansion or rupture [54,55].

Summary

The formation of a thrombus within a coronary artery with obstruction of coronary blood flow and reduction in oxygen supply to the myocardium produce ACSs. These thrombotic episodes largely occur in response to atherosclerotic lesions that have progressed to a high risk-inflammatory/prothrombotic stage by a process modulated by local and systemic factors. Although distinct from one another, the atherosclerotic and thrombotic processes appear to be closely interrelated as the cause of ACS through a complex multifactorial process named atherothrombosis. The cellular and molecular mechanisms at play in atherothrombosis are subject of extensive investigation. The identification of dangerous lesions by angiography is often rendered impossible

because, due to remodeling, their stenosis is mild. Advances in noninvasive imaging techniques will help recognize subclinical pathology, identify plaques at risk, and reduce the clinical impact of atherothrombosis, enabling us to better risk stratify the disease and perhaps to customize the treatment and directly monitor its effect.

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