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CORONARY HEART DISEASE (JA FARMER, SECTION EDITOR)

Pathophysiology of Acute Coronary Syndrome

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Abstract Despite improvements in interventional and pharmacological therapy for atherosclerotic disease, it is still the leading cause of death in the developed world. Hence, there is a need for further development of more effective therapeutic approaches. This requires better understanding of the molecular mechanisms and pathophysiology of the disease. Recent research in the last decade has changed our view of acute coronary syndrome (ACS): from a mere lipid deposition to an inflammatory disease; from ACS exclusively due to plaque rupture to the novel definitions of plaque erosion or calcified nodule; from the notion of a superimposed thrombus with necessary lethal consequences to the concept of healed plaques and thrombus contributing to plaque progression. In the hope of improving our understanding of ACS, all these recently discovered concepts are reviewed in this article.

Keywords Acute coronary syndrome · Pathophysiology · Atherosclerosis · Plaque rupture · Plaque erosion · Calcified nodule · Macrophage · Platelet · Inflammation · Innate immune response · Adaptative immune response

Introduction

In the USA alone, more than 400,000 Americans die annually of coronary artery disease, and more than 1,000,000 have acute coronary syndromes (ACS) [1]. Considering the increasing age and incidence of obesity and diabetes mellitus in the world population, it has been postulated that the morbidity from atherosclerosis and its clinical manifestations will increase, with a significant negative impact on the socioeconomics and quality of life of our society. This is reinforced because the community prevalence of ideal cardiovascular health (according to the American Heart Association) is only 0.1 % [2•], and those with worse cardiovascular health experience more ACS. Between 2010 and 2030, the total direct medical costs in the USA for cardiovascular diseases are projected to triple from $273 billion to $818 billion [3]. Although effective treatments are available, ACS still carry the burden of unacceptably high mortality and economic impact. With the aim of improving our understanding of the pathophysiology of ACS, this article provides a modern perspective on recent research regarding ACS, specifically reviewing the role of inflammation in atherosclerosis, how the pathologist's view of vulnerable plaque has recently changed, and the concept of vulnerable blood and advances in blood thrombogenicity.

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Process of Atherogenesis

Coronary atherosclerosis is the underlying condition for ACS, with very few exceptions. ACS are rarely caused by coronary dissection, arteritis, myocardial bridging, thromboembolism, or coronary vasospasm without obvious coronary artery disease. Coronary atherosclerosis is known to develop in childhood and adolescence, as evident from fatty streaks seen in the Bogalusa study of individuals who died of trauma or other noncardiac causes [4]. These initial fatty streaks progress during young adulthood; in fact, in an autopsy series in Olmsted County, 83 % of young adults (median age 36 years) who died of nonnatural causes already had coronary atherosclerosis and 8 % had obstructive coronary disease [5]. If we extrapolate the findings to the general population, most

individuals more than 40 years of age in our society already have early atherosclerotic lesions in the coronary arteries.

Under pathological conditions (risk factors, mechanical injury, etc.), the endothelium becomes dysfunctional, switching the formerly antiatherogenic endothelial atmosphere to a proatherogenic one. Endothelial dysfunction is characterized by a change in the pattern of synthesis and secretion of different substances by the endothelium (e.g., from the antiaggregant and vasodilatant nitric oxide and prostacyclin to the proaggregant and vasoconstrictor thromboxane). The dysfunctional endothelium has three consequences. First, it exposes adhesion proteins [such as selectin, intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule], which facilitates the activation of monocytes and their adhesion to the dysfunctional area. Second, it enhances the activation and aggregation of platelets; these activated platelets (by releasing the content of their α-granules) serve as inflammatory mediators, expressing various receptors interacting with leukocytes and the activated endothelium, perpetuating the pathological process. These proteins facilitate the homing and internalization of the circulating monocytes to the subendothelial space, where they become macrophages. Finally, there is an enhancement of the penetration of circulating lipids into the intimal layer. Cholesterol accumulation plays a central role in atherogenesis. Low-density lipoprotein (LDL) cholesterol binds to the proteoglycans of the subendothelial space, where it undergoes an oxidative process. Oxidized cholesterol is highly toxic, and as part of a mechanism of defense, it is phagocytosed by the vessel wall macrophages. The presence of the oxidized lipids triggers a series of proinflammatory reactions via different mediators [e.g., TNF-α, IL-1, monocyte chemoattractant protein 1 (MCP-1)], perpetuating the activation and recruitment of monocytes and macrophages and inflammatory cells. Macrophages, by engulfing the lipid material, become foam cells. Usually the lesion core is formed by foam cells, which are separated from the blood by vascular smooth muscle cells (VSMC) and collagen. Failure of macrophages to remove cholesterol from the vessel wall promotes its apoptotic death, releasing cholesterol to the vessel wall, inducing the apoptotic death of VSMC and, more importantly, inflammatory substances such as tissue factor (TF; making the lesion more thrombogenic) and matrix metalloproteases (enzymes which digest the collagen and create a thinner plaque cap), thus making atherosclerotic lesions more prone to rupture (the so-called vulnerable plaque).

Atherosclerosis and Inflammation: Role of Innate and Acquired Inflammation

Until recently, the dominant concept was atherosclerosis involving a mere lipid deposition process in the vessel wall, with reactive VSMC proliferation which created luminal stenosis. The advent of the cell biological era of atherosclerosis supplanted this simplistic concept, and modern research (mainly in the last decade) identified immune mechanisms in the pathogenesis of atherosclerosis. We will briefly provide an update of the role of inflammation as a key regulatory process that links multiple risk factors for atherosclerosis and its complications with altered arterial biology.

Through evolution, the inflammatory response has increased in complexity and efficacy. Inflammation is a defense mechanism against infections in particular and any tissular injury in general, while also playing a role in the repair of damaged tissues. There are two distinct types of inflammation, innate and acquired inflammation, and both of them are important in the pathophysiology of atherosclerosis and ACS.

Innate Immunity

The primitive arm of inflammation is called innate immunity. It consists of a primordial inflammatory response, a "fast but blunt" response to prespecified agents (on the order of hundreds), often with preformed mediators, natural antibodies (preexisting in the host without necessity of previous contact with the antigen), the complement system, and a family of cell receptors which respond immediately to the antigen with no need for previous "education" of the immune system. The monocyte–macrophage system and the polymorphonuclear leukocytes are the main cells implicated in innate immunity. The most important receptors are scavenger receptors (playing a key role in the phagocytosis of oxidized lipoproteins by the foam cell) and the Toll-like receptors (a family of pattern-recognition receptors that recognize microbial structures and products). These receptors trigger a complex intracellular signaling cascade that stimulates the production of proinflammatory cytokines and other inflammatory mediators.

Monocytes are the main component of the innate immune system, but are also involved in endogenous inflammatory processes. The critical role of monocytes has been confirmed in macrophage colony-stimulating factor knockout mice; this study proves that monocytes are active participants in the progression of atherosclerosis and not mere passive responders to intercurrent disease [6]. Monocytes can migrate from blood to tissues in response to signals and differentiate to inflammatory dendritic cells, macrophages, and foam cells (a critical step in atherogenesis).

There is also a large heterogeneity in the population of monocytes, according to their origin in humans or in animal models of atherosclerosis [7]:

1. *Human studies*: Passlick et al. [8] demonstrated in 1989 that human monocytes can be classified according to the expression of two specific surface receptors, the

lipopolysaccharide receptor (CD14) and the low-affinity FCγ receptor III (CD16).

(a) $CD14^{++}$ $CD16^{-}$ monocytes, also known as "classic" or Mon1, are the most frequent subtype, express CCR2 (receptor for MCP-1), CD62L, CD64, and CD115, but not CX3CR1, and are considered proinflammatory (by releasing myeloperoxidase) [9]. They depend on MCP-1 for migration.

(b) A second subtype is the $CD14^{+}$ $CD16^{++}$ monocyte (Mon3, also called "nonclassic" or resident); they do not express CCR2 or CD64, but express CX3CR1 (receptor for fractalkine). They seem to be important in reparative processes, appear late in acute inflammation, and depend on fractalkine for migration.

(c) A recently described subtype is the $CD14^{++}$ $CD16^{+}$ monocyte (Mon2, also called "intermediate"). They express high levels of CCR2, CX3CR1, and CD115, but not CD62. Their role is still not well understood but they may play an inflammatory role.

These subtypes have important prognostic importance. In a recent article analyzing 951 patients undergoing cardiac catheterization with a follow-up of 2.6 years, both the classic and the intermediate monocytes predicted worse outcome, but only the intermediate ($CD14^{++}$ $CD16^{+}$) monocytes were independently related to cardiovascular events, and might constitute a target cell population for new therapeutic strategies in atherosclerosis. Furthermore, systemic and local intrathrombus levels of myeloperoxidase are important markers of plaque vulnerability and thrombus formation [10] in plaque erosion.

2. *Mouse studies*: Two distinct monocyte subpopulations have been detected according to the expression of the marker Ly-6C (also known as Gr-1), Ly-$6C^{high}$ and Ly-$6C^{low}$. On the basis of the expression of the chemokine receptors CCR2 (receptor for MCP-1) and CX3CR1 (receptor for fractalkine), murine Ly-$6C^{high}$ ($CCR2^{high}$ $CX3CR1^{+}$) monocytes are similar to human $CD14^{high}$ $CD16^{low}$ monocytes (Mon1), whereas murine Ly-$6C^{low}$ ($CCR2^{-}$ $CX3CR1^{high}$) monocytes are similar to human $CD14^{low}$ $CD16^{high}$ monocytes (Mon3). This finding allows the study of the functional consequences of human monocyte heterogeneity in murine experimental models. Ly-$6C^{high}$ monocytes are potent inflammatory mediators, whereas Ly-$6C^{low}$ monocytes are important in the resolution of the inflammation [11]. In fact, hyperlipidemia increases the percentage of Ly-$6C^{high}$ monocytes [12]. Once recruited, the different types of monocytes exhibit different biological activities: Ly-$6C^{high}$ monocytes express TNF-α, IL-1, myeloperoxidase, matrix metalloproteases, and cathepsins and are proinflammatory, whereas Ly-$6C^{low}$ monocytes express IL-10, TGF-β, and proangiogenic vascular endothelial growth factor and play a reparative role [11].

Macrophage polarization creates two different subtypes, M1 and M2. M1 or "classic" macrophages are activated in the classic way (lipopolysaccharide or interferon-γ), and the markers of activation are CD80, CD86, inducible nitric oxide synthase, and CD11b. M1 macrophages release TNF-α, IL-1, and IL-6, thus being generally considered proinflammatory macrophages. M2 or "alternative" macrophages are activated in the alternative way (IL-4 or IL-13), and their markers of activation are mannose receptor (CD206), arginase 1, CD36, CD163, IL-10, FIZZ1, and YM1, and they secrete TGF-β. M2 macrophages are considered reparative or modulators of inflammation. It is usually considered that Ly-$6C^{high}$ monocytes preferably become M1 macrophages, whereas Ly-$6C^{low}$ monocytes become M2 macrophages. In humans, low expression of CD163 (i.e., reduced M2 polarization) is associated with more intraplaque hemorrhage, more oxidative stress, a greater inflammatory profile inside the plaque [13•], and more apoptosis of VSMC and macrophages [14]; in summary it is a more vulnerable phenotype of the plaque.

Besides the crucial contribution of macrophages to atherosclerosis, recent research has demonstrated an additional contribution of neutrophils to initial atherosclerosis development [15] and to plaque vulnerability [16]. Recent research has shown that mast cells are already deeply implicated in atherosclerosis [17] by releasing proinflammatory molecules and vasoactive mediators (histamine and leukotrienes); in fact, the inhibition of mast cells reduces the development of atherosclerosis [17].

Innate immunity also plays a role at the crossroads between thrombosis and inflammation. For instance, the prostaglandins produced by the cyclooxygenase 2 are both proinflammatory and prothrombotic; that is why cyclooxygenase 2 inhibitors can enhance the thrombotic risk. Besides, platelets store preformed proinflammatory cytokines in their α-granules, so they can easily be exteriorized in the platelet membrane; a paradigmatic example is CD40L (CD154), which facilitates the adhesion of activated platelets to the activated endothelial cells (expressing CD40), thus favoring the formation of thrombus on the atheroma plaque. Platelets also release additional inflammatory mediators, myeloid-related proteins 8 and14 [18]. These bind the monocyte receptor Toll-like receptor 4 (thus activating innate immunity [19]) and also induce endothelial cell apoptosis [20], thus favoring the creation of the superimposed thrombus.

Adaptative Immunity

The adaptative immunity response has a much more recent phylogenetic origin. In contrast to innate immunity, adaptative

immunity is slower (weeks), requires certain "education" of the immune system (by being previously in contact with the antigen), and displays exquisite specificity. The paradigmatic example is the immunity conferred by vaccines.

The role of adaptative immunity has been recognized recently. Lymphocytes interact with dendritic cells (antigen-presenting cells) and they initiate the immune response. Dendritic cells are very frequent in atheroma and in the lymph nodes of that anatomical region, and they present antigens to T cells with co-stimulatory molecules that incite this arm of immunity. The antigens more frequently causing adaptative immunity are components of oxidized lipoproteins. The clone of T cells that recognizes antigen in this context will proliferate to amplify the immune response. On renewed exposure to the specific antigen, these T cells produce cytokines and trigger inflammation, and some T cells have mechanisms specialized for killing cells.

Cellular Response (Tcells)

$CD4^+$ Lymphocytes These express CD4 in the membrane and recognize the antigens presented by dendritic cells. CD4 cells are the main lymphocyte subtype in atheromas and they play a key role in atherogenesis. CD4-knockout mice develop reduced atherosclerosis [21], whereas bone-marrow reconstitution with CD4 cells increases atherosclerosis [22]. It thus seems that CD4 cells are proatherogenic.

With regard to T helper (T_h) lymphocytes, the T_h1 lymphocytic response (which is similar to the M1 macrophage response) amplifies proinflammatory metabolic mechanisms by the release of cytokines and interferon-γ, thus aggravating atherosclerosis. T_h17 cells are a recently discovered subtype of T cells, and seem to be proinflammatory. The T_h2 response synthesizes cytokines, modulating the inflammation (such as IL-4, which promotes humoral immunity); the role of T_h2 cells in atherosclerosis is still under discussion, with some articles suggesting antiatherogenic roles [23], whereas other works suggest a role in aneurysm development [24]. We note that human atherosclerosis shows less marked T_h1 versus T_h2 polarization than murine models.

Regulatory T (T_{reg}) cells seem to modulate the development of atherosclerosis. In fact, the blockade of TGF-β, one of the main T_{reg} mediators, accelerates atherosclerosis [25].

In summary, T_h1 and T_h17 responses are proatherogenic, whereas T_h2 and T_{reg} responses seem to be antiatherosclerotic.

$CD8^+$ Lymphocytes Around 33 % of T cells in the atheroma plaque exhibit the membrane receptor CD8 and recognize antigens bound to the HLA complex on many cell types. The main role of CD8 cells is cytotoxic, i.e., destroying damaged cells by cell-to-cell contact. Therefore, CD8 lymphocytes can kill the VSMC constituting the fibrous cap of the plaque and the macrophages infiltrating the lesions, thereby promoting the destabilization of the plaque [26]. A specific subtype of T cells, natural T cells, react with molecules presented by CD1 molecules and induce a proinflammatory reaction [27].

Humoral Response (B Cells)

Several works suggest that humoral immunity attenuates atherosclerosis (in contrast with cellular immunity, which usually aggravates atherosclerosis). Splenectomy worsens atherosclerosis by eliminating the spleen, the reservoir of B cells [28]. Hypercholesterolemic mice develop a strong humoral response against specific epitopes of oxidized LDL [29]. On the basis of this reduced atherosclerosis severity when a humoral response is developed, a modern line of research consists in creating a "vaccine" against oxidized LDL as a measure to mitigate the atherogenic process.

Plaque Morphology Associated with ACS

Three classic plaque phenotypes are associated with ACS [30••, 31••]. From a review of the literature, including 22 autopsy studies in which 1,847 coronary arteries were explored microscopically with the purpose of identifying the underlying cause of thrombosis, it can be concluded that the great majority of coronary thrombi (73 %) developed on top of a ruptured atherosclerotic plaque [31••].

Plaque rupture is defined as a structural defect—a gap—in the fibrous cap that separates the lipid-rich necrotic core of a plaque from the lumen of the artery. A recent review [31••] found that plaque rupture was the main cause of coronary thrombosis regardless of the clinical presentation [myocardial infarction (MI), 79 %; sudden coronary death, 65 %], age (older than 60 years, 77 %; younger than 60 years, 64 %), sex (male, 76 %; female, 55 %), and continent (Europe, 72 %; USA, 68 %; Asia, 81 %).

An interesting concept is that clinically silent plaque rupture is not a rare phenomenon, and plaque rupture with mural thrombosis appears to be a common cause of episodic but asymptomatic progression to severe stenosis [32]. In fact, healed ruptures were found in 61 % of hearts and the percent areal luminal stenosis increased with increased numbers of healed sites of previous rupture. Therefore, we can conclude that the progression of atherosclerosis involves two distinct processes: a long one that leads to slow luminal narrowing (the "classic" concept of atherosclerosis), and a short one that causes rapid luminal obstruction (plaque hemorrhage with luminal thrombosis, healing of the plaque, and incorporation of the thrombus into the coronary plaque, thus narrowing the lumen and increasing plaque burden).

The prototype of ruptured plaque contains several pathological features : (a) a large and soft lipid-rich necrotic core

(usually more than 30 % of the plaque); (b) covering by a thin cap (less than 65 μm when assessed postmortem [33] or 49 μm if assessed by optical coherence tomograph (OCT) [34]); (c) inflamed fibrous cap (activated macrophages and T cells); (d) few VSMC; (e) positive remodeling mitigating luminal obstruction (mild stenosis observed by angiography); (f) neovascularization from vasa vasorum; (g) intraplaque hemorrhage; (h) adventitial/perivascular inflammation; and (i) a "spotty" pattern of calcifications.

In the 1990s the term "plaque erosion" was introduced to describe thrombosis without plaque rupture [35]. Plaque erosion is defined when serial sectioning of the thrombosed arterial segment fails to reveal plaque rupture. Typically, the endothelium is missing at the erosion site, and the exposed intima consists predominantly of VSMC and proteoglycans, but the blood does not come into contact with the lipid-rich necrotic core. In contrast with plaque rupture, the features of plaque erosion show the presence of pathological intimal thickening or a fibroatheroma with preserved and intact media, and with a lower severity of inflammation [33] and luminal stenosis [35] than ruptured plaques.

The mechanisms of superficial erosion have received much less attention than those involved in the rupture of the fibrous cap. Apoptosis of endothelial cells could contribute to their desquamation. Oxidative stress, in hypochlorous acid—the product of myeloperoxidase, an enzyme released by activated leukocytes associated with atheromas—can initiate apoptosis of endothelial cells [36]. These apoptotic cells are able to synthesize and release the procoagulant TF, thus propagating endothelial cell loss and local thrombosis in coronary arteries. The mechanisms of superficial erosion merit attention in future investigations; they are much less well understood than the mechanisms underlying the fracture of the plaque's fibrous cap.

The term "calcified nodule" applies to a rare type of coronary thrombosis not caused by plaque rupture but related to disruptive nodular calcifications protruding into the lumen [33]. These usually occur in older individuals and in tortuous heavily calcified arteries. Calcified nodules have distinct features identifiable by intravascular ultrasonography (IVUS; irregular, protruding, and convex luminal surface and irregular leading edge of the calcium), permitting their identification in vivo [37]. Interestingly, a recent analysis of the PROSPECT trial shows that the prevalence of calcified nodules was 17 % per artery and 30 % per patient; they were located less than 40 mm from the ostium of the coronary artery in 85 % of left anterior descending arteries and in 86 % of left circumflex arteries, whereas calcified nodules within the right coronary arteries were evenly and more distally distributed. Patients with calcified nodules were significantly older and had more plaque volume and more thick-cap fibroatheroma, but fewer nonculprit lesion major adverse events on follow-up. Surprisingly, the PROSPECT trial has found that calcified nodules, in spite of being associated with more plaque volume and older patients, were unlikely to cause coronary events during the 3-year follow-up, probably because they were also associated with more thick-cap fibroatheroma [38•].

This classification based on pathology has been confirmed by a recent OCT study analyzing 126 ACS patients who underwent preintervention OCT [39•]. The incidences of plaque rupture, erosion, and calcified nodule were 44 %, 31 %, and 8 %, respectively. ST-elevation MI patients exhibited plaque rupture more frequently (71 % vs 39 % in erosion and 0 % in calcified nodule). Patients with erosion were the youngest (54 years vs 61 years in plaque rupture and 65 years in calcified nodule), more frequently presented with non-ST-elevation ACS (62 % vs 29 % in plaque rupture), and exhibited the least severe stenosis (55 % vs 66 % in calcified nodule and 69 % in plaque rupture). Lipid plaque occurred less frequently in erosion (44 % vs 100 % in plaque rupture) and, when lipid was present in erosion, the plaque exhibited a thicker fibrous cap (169 μm vs 60 μm in plaque rupture) and a smaller lipid arc (203º vs. 276º in plaque rupture). Interestingly calcified nodule only appeared in non-ST-elevation ACS (100 % of cases).

Plaque rupture is a commoner cause of coronary thrombosis in men (80 %) than in women (60 %) [40–42], and plaque rupture is especially rare in premenopausal women [40]. There is a significant association between high cholesterol levels and thrombosis caused by plaque rupture (versus erosion) [40, 41]. Smoking seems to be more associated with plaque-erosion-promoted thrombosis rather than atherosclerosis [40, 41]. Fresher thrombi are more frequently associated with plaque rupture than erosion, suggesting that the former is more frequently associated with acute presentation [42].

A recent ambitious pathology study [43•] aimed at identifying the hierarchical importance of the histomorphometric components of the plaque, i.e., to define which component best identified plaque vulnerability. Thickness of the fibrous cap emerged as the best discriminator of plaque type; the cap thickness was less than 55 μm in all ruptured plaques (which was similar to the value of 49 μm measured by OCT [34]), and more than 85 μm in all stable, fibroatheroma lesions. Therefore, plaques smaller than 85 μm should be considered vulnerable and should alert clinicians. A second analysis focused on the plaque characteristics assessed by noninvasive imaging; as the thickness of the fibrous cap cannot be obtained by noninvasive imaging, it was excluded from this second analysis, and macrophage infiltration and necrotic core appeared as the two best discriminators of plaque types. This pathology study confirms the results of the recent prospective natural history study on plaque vulnerability, the PROSPECT trial [44••], wherein IVUS-based thin-cap fibroatheroma, plaque burden of more than70%, and luminal stenosis of less than 4 mm^2 were associated with adverse events during a 3.4-year follow-up.

Contrary to common perception, plaque rupture and thrombus formation most often do not lead to coronary events. Rather, both plaque rupture and thrombus formation are fairly frequent events that are instrumental in plaque progression and the development of lumen stenosis. In patients who died of noncardiac causes but had risk factors for coronary artery disease, 31 % of the patient population showed plaque ruptures [45]. A different group revealed multiple healed coronary arterial stenosis; in fact, only 11 % of plaque ruptures are virgin in nature, i.e., they were not preceded by prior ruptures of the same plaque in these studies [32]. Finally, clinical studies using angioscopy or IVUS found that 80 % of patients with ACS showed evidence of thrombus and plaque rupture remote from culprit sites [46], further confirming its frequent presence despite the lack of events originating from nonculprit sites. All of the above-mentioned observations strongly suggest that an acute coronary event is not a necessary consequence of coronary plaque rupture but rather is an unusual correspondence of a plaque rupture or erosion. Most commonly, plaque ruptures or erosions occur without symptoms and lead to progression of plaque volume. These observations suggest that identifying plaques that are prone to rupture, i.e., vulnerable plaques, may not be as significant as commonly perceived.

This concept was recently confirmed in the PROSPECT study, which followed up 697 patients after virtual histology IVUS for 3 years for the occurrence of adverse cardiac events. Although 595 thin-cap fibroatheromas were identified by IVUS in 313 of 623 patients, only 26 of these plaques were sites of subsequent events at 3 years. Indeed, of more than 3,000 nonculprit lesions identified by IVUS at the baseline in 673 patients with ACS, only six were subsequently related to MI and death after 3 years. This suggests that the identification of a potentially vulnerable plaque may confer some increase in coronary event risk but that it is far less than generally assumed.

An interesting feature is the dynamic nature of acute coronary plaque morphology, which may further reduce the significance of identifying plaque characteristics at a given point in time. In a clinical study using IVUS in 99 stable patients [47], 75 % of thin-cap fibroatheromas (believed to be responsible for ACS) stabilized or healed to form thick-cap fibroatheromas during 1-year of follow-up, and there was no difference in the baseline composition between lesions which stabilized and lesions which remained thin-cap fibroatheromas. Conversely, some thick-cap fibroatheromas and intimal thickening at the baseline changed into thin-cap fibroatheromas over 12 months. If confirmed, these data suggest that a plaque that appears vulnerable at a given time may be less vulnerable just months later, whereas another plaque, initially not vulnerable, may have developed vulnerable characteristics within the same time frame. It is interesting that this study [47] was performed on stable coronary artery disease patients, whereas a more recent study did not confirm these findings in the setting of ST-elevation MI patients [48•]. In fact, in a subanalysis of the HORIZONS-AMI trial regarding untreated nonculprit lesions evaluated by IVUS, only 22 % of plaques evolved into a stabler phenotype, and most lesions additionally progressed (reduction in lumen area and increase in necrotic core) [48•].

Beyond Plaque Rupture: Thrombus Formation and "Vulnerable Blood"

As mentioned before, coronary atherosclerosis is a prerequisite for ACS, it is a necessary condition, but not a sufficient one. For instance, we have learned in recent years that the severity of coronary lesions does not correlate with the clinical occurrence of ACS: indeed, more than 70 % of patients with ACS have a culprit coronary lesion less than 50 % of the luminal diameter of angiography. Therefore, factors other than the mere presence of a coronary atherosclerotic lesion need to be involved for an ACS. The thrombogenic potential of the circulating blood may play a key role.

After plaque rupture/erosion, the subendothelial space [containing TF, collagen, and von Willebrand factor (vWF)] is exposed to the blood flow. Specifically, first, TF interacts with circulating factor VII, which activates factor X, resulting in the ulterior conversion of the inactive zymogen prothrombin into the active enzyme thrombin. Besides, under conditions of high shear stress, as those found close to a significant stenosis, vWF plays a critical role in mediating platelet adhesion via glycoprotein Ibα. After adhesion, platelets undergo a remarkably complex series of morphological and biochemical changes, leading to the generation and release of soluble mediators, including thromboxane A2, ADP, and serotonin, which in turn cooperatively promote further activation and recruitment of additional platelets from the circulation; this results in upregulation of glycoprotein IIb/IIIa, also named integrin $\alpha_{IIb}\beta_3$, which is capable of binding multiple ligands, including vWF, fibrinogen, fibrin, and fibronectin, and is fundamental for the formation of stable platelet aggregates. In addition to the formation of the initial hemostatic plug, platelets also possess a procoagulant function, as they provide a catalytic surface for the optimal assembly of coagulation factors. Thrombin, on the other hand, not only has the ability to generate fibrin polymers, but is also the most potent platelet activator by binding platelet protease-activated receptor. Another pathophysiological role of platelets is the cross talk with leukocytes. Recruitment of monocytes to vessel-wall-bound platelets can occur via interaction of platelet P-selectin with its cognate receptor P-selectin glycoprotein ligand 1 on leukocytes; this interaction is important for the propagation of inflammation at the site of vascular injury, as well as for sustaining thrombus growth.

The concept of high blood thrombogenicity of hypercoagulable state can be explained by the fact that TF is not only present in the subendothelium, but is also in a circulating state in the blood. Since the initial report by Giesen et al. [49] in 1999, evidence has been accumulated, and the prevailing hypothesis is that the circulating TF may have different origins. The first pool is associated with macrophages and platelets. Most of the TF molecules located on the cell surface have low activity (a phenomenon defined as encryption [50]); exposure of phosphatidylserine in response to various stimuli is considered the most potent inducer of TF "decryption" (activation). The second pool is associated with microparticles; these are submicron membrane vesicles released by different cell types as a consequence of activation or apoptosis [51•]. This prothrombotic effect is related to the provision of a phospholipid surface for assembly of coagulation factors, resulting in the amplification of the coagulation cascade. Microparticles are indeed very frequent in the atherosclerotic plaque, originating from macrophages, erythrocytes, and smooth muscle cells, whereas circulating microparticles derive mainly from platelets [52]. Quite importantly, it has been shown that microparticles present in atherosclerotic plaques may transfer adhesion molecules, such as ICAM-1, to endothelial cells, possibly causing a further recruitment of inflammatory cells [53•]. Circulating microparticles bear TF at their surface, representing an important source of the so-called blood-borne TF [51•]. These mechanisms may lead to an increased concentration of TF at the site of thrombus formation, which is believed to initiate and accelerate blood coagulation and fibrin formation.

Finally, we wish to point out the interesting role of TF in the cross talk between inflammation and coagulation. First, TF can mediate coagulation-independent biological phenomena, including angiogenesis [54] (known to be a crucial component of the vulnerable plaque), adhesion of monocytes to the endothelium [55], and proliferation of cells inside the atherosclerotic plaque via the extracellular-signal-regulated kinase pathway [56]. Conversely, inflammation may enhance the thrombogenic status of the blood; for instance, VSMC and endothelial cells express functional TF on their membranes following stimulation with C-reactive protein [57] or oxygen free radicals [58], turning their phenotype into a prothrombotic one.

Conclusion

We have reviewed the new paradigms regarding the pathophysiology of ACS, namely, the role of innate immunity in atherosclerosis (monocyte and macrophage polarization), the influence of adaptative immunity in the maintenance of the atherogenic process, the new concepts of plaque erosion and plaque rupture according to the pathology findings, and the importance of blood thrombogenicity (TF-vulnerable blood) for ACS. This last decade has brought promising developments and sobering realizations regarding the pathophysiology of ACS.

Compliance with Ethics Guidelines

Conflict of Interest Carlos G. Santos-Gallego, Belen Picatoste, and Juan José Badimón declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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