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Review

Overview of Atherosclerotic Plaque: From Formation to Complication

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Abstract: Atherosclerosis is a serious worldwide health problem. The pathogenesis of this disease is complex and includes a multilevel interaction of such processes as inflammation, endothelial dysfunction, mitochondrial dysfunction, and multiple modifications of lipid particles. With the increase in life expectancy, mankind is more and more often faced with the unpleasant consequences of atherosclerosis. Atherosclerosis is a precursor to serious health concerns, including cardiovascular disease, leading to disability and even death. Thrombosis is one of the possible complications of atherosclerosis. The cause of atherothrombosis is the rupture of an atherosclerotic plaque and subsequent aggregation of platelets. In this place, a blood clot forms, which in a short time completely blocks the blood flow through the vessel. Sometimes pieces of plaque break off and travel through the arteries with the blood flow. If such a blood clot, which is a piece of plaque, gets stuck in a vessel and blocks blood flow, for example, in a heart muscle, it can cause angina pectoris and myocardial infarction. In this review, we have collected a detailed description of the processes of atherogenesis from the very beginning to the immediate formation of a thrombus. To collect the relevant data, we searched the PubMed database, paying special attention to the systematic reviews and *in vivo* studies. All researches considering cardiovascular diseases have to be ethically performed, which implies patient consent and animal studies ethics.

Keywords: Atherosclerosis, Thrombosis and Plaque Formation

Introduction

There are several stages of atherosclerosis pathogenesis. Studies conducted in both humans and animals demonstrate that fatty streaks act as the first indicator of atherosclerosis. As a rule, primary lesions occur due to the focal growth of lipoproteins in the intimate layer of the arteries (Rafieian-Kopaei *et al*., 2014).

Proteins, phospholipids, and lipids, including cholesterol and triglycerides, combine to form lipoprotein particles. Among these, LDL, with its high cholesterol content, is a crucial factor in atherosclerosis (Feingold, 2015).

Due to its ability to enter the endothelium or cling to the components of the extracellular matrix (like proteoglycan, for example), lipoprotein can pile up in the intima of blood vessels (Linton *et al*., 2019; Summerhill *et al*., 2019).

An imbalance between the various components of the matrix may occur at the site of the lesion. For example, if there is a relative elevation in heparan sulfate molecules in three key groups of proteoglycans compared to keratan sulfate and chondroitin sulfate, this is capable of causing lipoprotein adhesion, which will further lead to inhibition of the process of quitting intima and, as a consequence, to their speeded-up accumulation (Tran-Lundmark *et al*., 2008).

In the early development of atheroma, plaques tend to expand outward from the vessel, indicating a predisposition for atherosclerotic vessels to widen. Once the plaque encompasses over 40% of the inner elastic layer of the vessel, the arterial passage is considered restricted (Rafieian-Kopaei *et al*., 2014). At the end of the life of the plaques, a restrictive obstacle to blood flow emerges.

The results of numerous studies suggest that atherosclerosis is the outcome of intimal damage

involving specific cellular reactions, including monocytes, SMC, and lymphocytes.

The early soft lesion is identified by the existence of foam cells, extracellular fat deposits, and a minimal number of platelets. Progressing further, SMCs undergo multiplication, culminating in increased bleeding into the plaque in the final stages (Xu *et al*., 2019). The brief summary of atherosclerosis development is represented in Fig. 1.

LDL-C Trapping

The development of atherosclerosis begins with the trapping of lipoprotein in the site of the lesion (Rafieian-Kopaei *et al*., 2014; Khatana *et al*., 2020).

Distinct modifications to LDL particles, notably oxidation, play a pivotal role in their absorption. The oxidation process enhances the affinity of LDL for CD36 and SR-A, scavenger receptors responsible for facilitating the macrophage uptake of oxidized LDLs.

Normally, there is a harmony between the level of LDL in plasma and the internal concentration of LDL in arterial walls. An elevation in plasma lipid levels causes a noteworthy accumulation of these particles in the intima (Steffen *et al*., 2021). This occurs because of an increase in extracellular proteoglycans, which possess a strong attraction for LD (Little *et al*., 2002; Williams and Tabas, 1995). Since there is a direct correlation between the concentration of serum LDL and the number of lipoproteins retained in the lesion, its level in the blood is considered an indicator of atherogenesis (Boren *et al*., 2020). In the initial phases of atherosclerosis, lipids gather within the extracellular matrix, creating a lipid-proteoglycan-rich structure enveloped by VSMCs. There, towards the media, biglycan, proteoglycan of the extracellular matrix, contributes to the binding, accumulation, and storage of LDL-C. At this stage, the inner part of the subendothelial layer, just below endothelial cells, is still poor in VSMCs and biglycan and is free from lipoprotein deposition (Hurt-Camejo and Camejo, 2018).

It is now assumed, that one of the key initial events in atherogenesis is endothelial injury. This can happen in the endothelium surrounding the lumen of the mother vessel in the endothelium of vasa vasorum, or both. There are two main theories about the role of the endothelial dysfunction. According to one of them, called response-to-retention, in response to predisposing stimuli (mechanical strain and cytokines), the initial event is the retention of lipoproteins bound to the ECM in the intima (Sedding *et al*., 2018). Lipoproteins enter the arterial wall through dysfunctional endothelium that surrounds the lumen of the vessel and this process is followed by the entry of monocytes and other inflammatory cells. The second one, the response-to-injury hypothesis, states that an initial injury (mechanical injury or toxins) leads to endothelial dysfunction and the passage of inflammatory cells, especially macrophages, and T-cells, into the arterial wall, followed by the proliferation of VSMCs (Mundi *et al*., 2018).

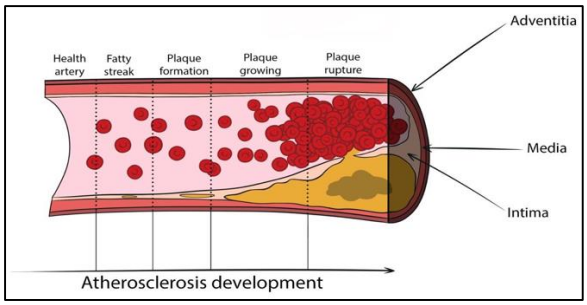

Fig. 1: Atherosclerosis development. Atherosclerotic plaque evolves through various stages, starting with the formation of fatty streaks and progressing to plaque rupture and thrombus formation. The process of fatty streak formation involves four steps: (1) Trapping of LDL-C; (2) Activation of endothelial cells; (3) Activation of leukocytes and (4) Formation of foam cells

The trapping of low-density lipoproteins leads to an elevation of LDL concentration in the intima, as well as an increase in the duration of their stay in the lesion. Both of these factors result in spontaneous oxidation and cellular oxidation of trapped particles (Kattoor *et al*., 2019).

Endothelial Cells Activation

Cytokines and oxidized lipids are extremely important for endothelial cell activation. In the initial phases of atherosclerosis, monocytes and T-lymphocytes invade the vessel's intima (Wu *et al*., 2017; Chistiakov *et al*., 2015a-b).

Simultaneously, in LDL oxidation, adhesion, and absorption molecules play significant roles. Monocyte differentiation into macrophages, essential for foam cell formation, involves the uptake of modified lipids like Oxidized LDL (Ox-LDL) (Seo *et al*., 2015). This process depends on receptor expression, purifying enzyme secretion, and various cytokines. Ox-LDL activates T-cells, acting as an antigen and secreting cytokines that activate macrophages and induce changes in endothelium and SMC (Gao *et al*., 2021; Sobenin *et al*., 2014).

Small, LDLs can pass through the endothelial barrier and attach to proteoglycans via apolipoprotein B100 to stay in the subendothelial space. This LDL undergoes oxidation (ox-LDL) and triggers various pro-inflammatory conditions through a receptor called Lectin-like Oxidized LDL receptor-1 (LOX-1). The increased expression of Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular-Cell Adhesion Molecule-1 (VCAM-1) caused by ox-LDL promotes the adhesion of monocytes and inflammatory cells to the endothelium. Oxidized LDL particles induce the release of Monocyte Chemotactic Protein-1 (MCP-1) and Monocyte Colony-Stimulating Factor (M-CSF) from

endothelial cells and smooth muscle cells, both acting as attractants for monocytes. Ox-LDL also leads to an increase in Reactive Oxygen Species (ROS) and a decrease in nitric oxide production. Monocytes mature into macrophages and express Scavenger Receptors (SRs), Cluster of Differentiation 36 (CD36), LOX-1 and Toll-Like Receptors (TLRs). The interaction between ox-LDL and CD36 prompts monocyte maturation, macrophage activation, and macrophage retention, while macrophage SRs enhance the uptake of ox-LDL and the formation of foam cells. The accumulation of ox-LDL promotes apoptosis in foam cells and initiates inflammatory progression. Ox-LDLs also stimulate SMCs to increase the expression of growth factors like Platelet-Derived Growth Factor (PDGF) for migration and basic Fibroblast Growth Factor (bFGF) for proliferation. The proliferation of SMCs contributes to the thickening of atherosclerotic plaques and the formation of a necrotic core. The interaction between ox-LDL and CD36 in resting platelets results in platelet aggregation and activation. Activated platelets express LOX-1, which promotes adhesion to endothelial cells and enhances the release of endothelin-1. This impairs endothelial function, reduces production of NO, and increases prostaglandin synthesis.

Platelet Adhesion to the Dysfunctional Endothelium

Arterial wall inflammation alters the normal functioning of the endothelium and launches the platelet and leukocyte recruitment at the initial phase of atherosclerotic lesion formation. According to modern understanding, the recruitment and adhesion of platelets can launch and support the chronic inflammatory processes that contribute to atherosclerotic lesion formation.

The activation of platelets and their accumulation at the arterial wall is further increased by the impairment of NO, PGI2, and endothelium-derived platelet inhibitors production (Busse *et al*., 1993) as well as by expression of proinflammatory mediators on dysfunctional endothelium at early stages of atherogenesis (Zibara *et al*., 2000).

P-selectin plays a role in the temporary adhesion of platelets to the endothelium (Frenette *et al*., 1995) whereas PECAM contributes to the stable adhesion (Rosenblum *et al*., 1996). Furthermore, endothelial cells express various adhesion and platelet-activating molecules on their surface, including chemokines, selectins (P-, E-selectin), and Cellular Adhesion Molecules (VCAM, ICAM, PECAM) (Wagner and Frenette, 2008).

Platelets can adhere even to the inflamed endothelium without of endothelial disruption (Massberg and Messmer, 1998). This adhesion is mediated by platelet aIIbb3 and GPIba, as well as endothelial ICAM-1 and avb3 integrin interactions (Massberg *et al*., 2005). The role of molecular bridges in aIIbb3-mediated adhesion is played by fibronectin, VWF, and fibrinogen (Bombeli *et al*., 1998). Endothelium-bound Fractalkine (CX3CL1) has been shown to activate adherent platelets *in vitro* (Schulz *et al*., 2007).

Leukocytes Activation

The initiation of leukocyte recruitment, whether in infectious or non-infectious diseases, begins with the activation of inflammatory tissue. This activation is an inherent reaction of the immune system to varied stimuli, encompassing tissue injury, cellular demise, pathogens, or toxic substances.

In early atherosclerosis, immune cells breach the endothelium, expressing adhesion molecules and chemokines (Doukas and Pober, 1990). Pro-inflammatory cytokines activate this process, involving TNF-α. Attraction molecules guide leukocyte migration (Mussbacher *et al*., 2019). Excessive MCP-1 expression induces monocyte migration, prevalent in atherosclerosis stages (Aiello *et al*., 1999). Ox-LDL regulates adhesion molecules and MCP-1 expression (Sawada *et al*., 2020).

During heightened inflammation, cells in the tissue recognize preserved Pathogen-Associated Molecular Patterns (PAMPs) and internal stress signals called Damage-Associated Molecular Patterns (DAMPs). These signals prompt the release of pro-inflammatory cytokines and chemokines. Endothelial Cells (ECs) respond by upregulating adhesion molecules and chemokines. This process involves rapid translocation of preformed molecules (type I activation) and slower, longer-lasting activation (type II). The expressed chemokines, including CCL2 and CXCL1, attract leukocytes through chemotaxis. Tissue-resident leukocytes, especially macrophages, release chemotactic molecules like CCL3. Activated platelets deposit chemokines like CCL5 and CXCL4 on ECs, enhancing leukocyte chemotaxis to inflammatory sites. Specificity in chemotactic molecules and their receptors recruits distinct leukocyte subsets.

Foam Cell Formation

After introduction into the intima, mononuclear phagocytes differentiate into macrophages.

Phagocytes contribute to averting atherosclerosis by ingesting lipids from the extracellular space. Certain macrophages that accumulate lipids can exit the artery wall and release lipids. If the influx of lipids into the artery wall surpasses their efflux (via phagocytes or other pathways), it can lead to lipid build-up and an increased likelihood of atheroma formation (Moore *et al*., 2013).

Macrophages take up and store modified LDL through scavenger receptors, transforming into foam cells. These receptors are located on the exterior of macrophages, endothelial cells, fibroblasts, and smooth

muscle cells. The manifestation of these receptors rises as monocytes transform into macrophages, driven by cytokines and oxidized lipids. Furthermore, the macrophage colony-stimulating factor enhances their expression (Aiello *et al*., 1999; Poznyak *et al*., 2020).

Within the plaque, macrophages acquire lipoproteins from their surrounding environment. This uptake can happen through scavenger receptors known as Pattern Recognition Receptors (PRRs) located on the surface of macrophages. Several types of these scavenger receptors exist, including Scavenger Receptor A1 (SR-A1), MARCO, CD36, SR-B1 and LOX1. These receptors are responsible for taking up oxidized forms of LDL (ox-LDL), which are produced due to increased oxidative stress in the artery wall. It's important to note that these scavenger receptors, functioning as PRRs, serve a broader purpose beyond lipid uptake. For instance, SR-A1 has been associated with regulating macrophage proliferation within the lesion, thus influencing macrophage numbers. CD36 has been linked to inflammasome activation, macrophage polarization, and the promotion of apoptosis and inflammatory gene expression. Additionally, LDL can be engulfed by macrophages via pinocytosis at high lipid concentrations and lipolytic enzymes present in the intima can generate modified forms of LDL, which are taken up by macrophages through scavenger receptor-independent pathways. Under normal circumstances, lipids taken up by macrophages are typically processed and effluxed from the cells, preventing the formation of foam cells as described earlier. However, in cases of dyslipidemia, excessive lipid uptake by macrophages due to limited negative feedback leads to defective cholesterol trafficking, impaired lipid efflux, and the formation of foam cells laden with lipids. This, in turn, affects macrophage phenotype and compromises immune functions.

Notably, during the formation of foam cells, lipid droplets in macrophages form not in the membrane of ER, but in the lysosomes. Usually, the cells of intima lose contact with each other and store lipids in lysosomes rather than in ER, turning into foam cells filled with lipid droplets formed from lysosomes. The widespread way of lipid granule formation is the transformation of lipids from smooth ER into lipid droplets. It is an important issue, which is discussed in the review by Mironov *et al*. (2020); Mironov and Beznoussenko (2022).

The surface ligand of Ox-LDL, which provokes its uptake by macrophage scavenger receptors, is phospholipids in the structure of Ox-LDL, oxidizing in the second place and establishing aldehydes capable of attacking lysine residues of APOB.

When the concentration of foam cells on artery walls becomes excessive, it triggers the formation of fatty streaks (Afonso and Spickett, 2019).

Some foam cells in less damaged areas undergo apoptosis, contributing to a lipid-rich necrotic core in severe atherosclerotic plaques. Monocytes, unlike other foam-producing cells, can generate harmful substances, causing significant damage to the endothelium, strong LDL oxidation, and substantial metabolic changes (Rafieian-Kopaei *et al*., 2014; Martinet *et al*., 2019).

Atheroma Formation

SMCs in atherosclerotic plaques, originally identified by contractile proteins, have been found to lose typical SMC markers in recent studies, often referred to as SMC-derived cells.

The migration of SMCs from the media to the intima, as well as their proliferation, is regulated by various growth factors produced by macrophages, Endothelial Cells (ECs), and T-cells. SMCs also possess Scavenger Receptors (SRs) on their cell surface and can take up modified LDL to form foam cells. Cytokines can modulate this process by influencing the expression of SRs either independently or in synergy with growth factors. Additionally, IL-1β disrupts the feedback regulation of LDL receptor mediated by cholesterol in these cells, leading to increased expression of this receptor.

Misra *et al*. (2018) found that some medial SMCs move into the intima, proliferate, and become fibrous cap SMCs. Cap SMCs generate descendants that enter the core, downregulate markers, and promote lesion growth. Alencar *et al*. (2020). discovered that two-thirds of SMC-derived cells express LGALS3 or pass through an intermediate stage. Cap SMCs skip this stage, indicating their origin from medial SMCs without transitioning through highly transformed phenotypes in the plaque's center. Vascular tissue sustains damage when neighboring SMC and endothelial cells release peptides like IL-1 and TNF, prompting SMC migration to the vessel wall's luminal side (Lim, 2019).

In this state, the migration of smooth muscle cells and the synthesized extracellular matrix form a fibrous cap. The fibrous cap consists of collagen-rich fibrous tissues, SMC, macrophages, and T-lymphocytes, which together create a mature atherosclerotic plaque that protrudes into the canal and interferes with normal blood flow in the vessels (Basatemur *et al*., 2019).

Macrophages and T-lymphocytes are found within the boundaries of a developed plaque. Macrophages secrete meta-proteinase, which favors the lysis of the extracellular matrix; and T-cells produce TNF-α, which helps to avoid collagen synthases in SMC (Ohmura *et al*., 2021).

These processes lead to the weakening of the plaque-shaped fibrous cap and can destroy it. The destruction of the fibrous cap outputs collagen and lipids into the bloodstream, which consequently results in the accumulation and adhesion of platelets, as well as in the formation of blood clots, which can unexpectedly stop blood flow (Periayah *et al*., 2017).

The Process of Formation of the Plaque

Atherosclerosis development results from an interplay of systemic risk factors, disruptions in shear stress, and the vascular wall's biological response. Refer to Fig. 2 for a visual representation.

The atherogenic phenotype of the endothelium has elevated permeability to circulating low-density lipoprotein and their high concentration in the tunica intima characterizes the initial phase of plaques formation. It was revealed that within the bloodstream, LDL particles can undergo a variety of modifications, such as oxidation, charge change, desialylation, and others. It is proposed that the risk of atherosclerosis development depends not on the total content of LDL in the blood but on the level of multiply modified LDL. That allows us to suggest that the level of multiply modified LDL is a better biomarker of atherosclerosis in comparison to the total LDL level.

Oxidation turns LDL into oxLDL, damaging the endothelium and activating inflammation through PPRs (Gillotte-Taylor *et al*., 2001). Cellular and humoral elements, along with factors from the environment and adventitia, contribute to the disease's progression by forming microvasculature within the plaque (Seiler *et al*., 2020). The damaged endothelium's activated Expresses Cytokines (ECS) chemokines and adhesion molecules, attracting monocytes to the atherosclerotic lesion and promoting their maturation into proinflammatory Macrophages (M1 phenotype) (Pircher *et al*., 2019).

Atherosclerotic plaques predominantly form at the branch points of arteries or at the inner curvature. These regions often have disturbed blood flow and the mechanical forces associated with this disturbance often affect the endothelium of the arteries. The shear stress usually causes anti-atherogenic gene expression and signal transduction profile that is lost at sites of disturbed blood flow. Moreover, ECs at the sites of impaired blood flow demonstrate the morphological changes, the permeability to macromolecules such as LDL appeared to be enhanced, extracellular matrix tends to accumulate. This causes the retention of such particles. Cytokines can modulate EC permeability. Thus, IFN-γ and TNF-α lead to the reorganization of the actin and tubulin cytoskeletons in ECs, thereby opening up gaps between adjacent cells. Activated endothelial cells release various chemokines, stimulating the recruitment of immune cells from the circulation, especially T lymphocytes and monocytes. Moreover, endothelial cells express ICAM-1, VCAM-1, and other adhesion proteins, which are also essential in immune cell recruitment.

Macrophages usually control lipoprotein metabolism by controlling LDL levels and cholesterol levels to support cholesterol homeostasis. Macrophages express Scavenger Receptors (SR) on their surface, which bind to ox-LDL, making it possible to absorb proteins in the cell (Sukhorukov *et al*., 2020).

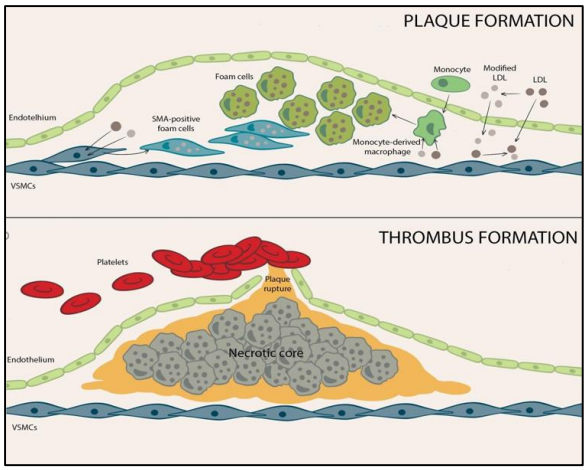

Fig. 2: Atherosclerotic plaque formation (above) and thrombus formation (below)

Macrophages express crucial enzymes like ACAT1, essential for cholesterol ester formation. These enzymes break down cholesterol esters into FAs and cholesterol. ABCA1, ABCG1, and SR-BI facilitate the transport of free cholesterol outside the cell. Atherosclerosis alters this, leading to cholesterol buildup and reduced expression of carriers. Foam cells result from uncontrolled accumulation of modified LDL and cholesterol esters in macrophages, triggered by inflammation (Dubland and Francis, 2015).

The initial immune response transitions into an adaptive response, involving T and B cells. Adaptive immunity detects molecules through BCRs and TCRs. T cells, with coreceptors like CD4, CD8, or CD3 linked to TCR, provide intracellular signaling upon recognizing an antigen-presenting cell. Naïve T cells differentiate into various T-cell types in plaques or lymphoid organs. Th1, the most common T-cell in atherosclerosis, responds to oxLDL stimuli, inducing atherosclerosis. Th2, though less significant, appears defensive, suppressing Th1 cells. ApoE−/−/IL-4−/−mice show a significant decrease in plaque size, prompting further exploration of hypothetical atherosclerotic Th2. Th17 and NKT cells possess both pro- and anti-atherogenic properties, requiring additional studies (Wondimu *et al*., 2010).

Treg, or regulatory T-cells, exhibit atheroprotective behavior by releasing IL-10 and Transforming Growth Factor β (TGF-β), contributing to immunomodulation. B-cells serve as antigen-presenting cells for T-cells and produce antibodies, influencing the immune response. B1 cells protect against neurodegeneration by inhibiting oxLDL absorption by macrophages. Conversely, B2 cells worsen atherosclerosis by releasing autoantibodies and cytokines, intensifying Th1 cells and macrophage activation. Throughout atherogenesis, Th1, Th17, Th2, and B-cells increase, while Treg decreases steadily.

In atherosclerotic plaques, CD4+ Th1 cells dominate, followed by CD8+, Th2, Treg, Th17, and NKT cells to a lesser extent. All Treg subtypes, including Foxp3+ Treg and Type 1 regulatory t cells (Tr1), demonstrate atheroprotective effects by inducing IL-10 and TGF-β, contributing to cell-mediated suppression.

At this moment, if not collapsed, foam cells pile up inside the plaque and, together with macrophages, increase the inflammatory signaling. This is achieved due to the release of chemokines and cytokines, which include IL-1, IL-6, TNF-α, and IFN-γ, as well as due to the production of reactive oxygen species, growth factor, and vascular smooth muscle cell proliferation, thereby speeding up the atherosclerosis development (Ramji and Davies, 2015).

In particular, the atheroma plaque consists of the following components: (1) Necrotic lipid nucleus formed from foam cells that are dead; (2) Circulating inflammatory and immune cells; (3) Endothelial and SMCs; (4) Detritus and connective tissue elements; (5) As well as the fibrous membrane covering the plaque.

Immunity and inflammation play crucial roles in the development and complications of atherosclerosis. Biomarkers of inflammation are recognized as independent risk factors for cardiovascular events. Thrombotic complications in atherosclerosis occur when the fibrous cap, surrounding the necrotic nucleus, ruptures into the vessel lumen. This disintegration is a result of proteolytic enzymes and heightened immune and inflammatory activities within the plaque. Consequently, it destabilizes the plaque, increasing the risk of rupture and thrombosis (Wolf and Ley, 2019).

Plaque Development

Invasive coronary angiography is a benchmark for assessing coronary artery disease and determining treatment strategies, both the development and regression of the lesion were generally considered as a change in the degree of angiographic lumen stenosis. However, since plaque rapture is considered a key ground of most medium and high-risk diseases, the pathology of the plaque is the main factor in acute events. Thus, the emphasis on increasing the degree of lumen stenosis appears to be unreasonable (Sun and Xu, 2014).

Intravascular ultrasound (Gogas *et al*., 2011) and computed tomography angiography (Cao *et al*., 2019) research has revealed that key indicators of CV events and plaque rupture include a thin fibrous membrane, the volume of the necrotic nucleus and positive remodeling.

Traditionally, high-risk plaque features were binary or categorized based on the absence of 1, 2, or 3 such features. Modern CTA studies stress the importance of quantifying these features for accurate assessment, given their interdependence and impact on prognostic significance for ischemia and future events (Baradaran *et al*., 2017). Among them, the volume of the necrotic core, identified by low attenuation on CTA, is crucial. An enlarged necrotic core weakens the fibrous cap, compromises vasodilatory capacity, and raises the risk of rupture, irrespective of the lumen stenosis degree (Ohayon *et al*., 2008).

The progression or regression of a plaque, rather than the percentage of lumen stenosis, is crucial for assessing rupture risk. For instance, an increase in necrotic core volume, positive remodeling, and fibrous membrane thinning (regardless of lumen changes) signifies plaque progression (Stefanadis *et al*., 2017). Conversely, a reduction in necrotic core volume, coupled with increased fibrous cap thickness and calcification (despite moderate lumen stenosis from negative remodeling), indicates plaque regression (Costopoulos *et al*., 2017).

Plaque Rupture

The specific mechanism behind plaque rupture remains unknown; however, it involves several factors such as thinning of the fibrous cap, increased levels of inflammatory cytokines and proteases, degradation of the extracellular matrix, decreased collagen synthesis, and the presence of injured or apoptotic cells within the necrotic core. All cell types involved in the development of atherosclerotic plaque are also implicated in plaque rupture and subsequent thrombosis. Molecular mediators associated with atherosclerosis can alter collagen metabolism, leading to thinning or weakening of the fibrous cap. Particularly, IFN-γ has been found to significantly inhibit the expression of genes encoding procollagens in smooth muscle cells, establishing a significant link between inflammation and impaired collagen synthesis in atherosclerotic lesions. Inflammatory cells within the plaque release various molecular signals, including cytokines, growth factors, tissue factors, IFN-γ, Matrix Metalloproteinases (MMPs), and Reactive Oxygen Species (ROS). Macrophage-derived foam cells secrete cytokines, while lymphocytes secrete CD-40L, among others.

Accumulation of free cholesterol within the plaque can induce apoptosis of macrophage-derived foam cells, as well as apoptotic cell death of SMCs and T-cells within the lesions. The release of cellular contents from apoptotic cells initiates the formation of the necrotic core, which is composed of lipid-rich material surrounded by fibrous tissue.

Excess extracellular cholesterol can form cytotoxic crystals, progressing atherosclerotic plaques into complicated atheromas, potentially causing coronary artery branch occlusion.

The persistent inflammatory response ultimately contributes to the destabilization of atherosclerotic plaques through the actions of proinflammatory cytokines. Studies have indicated that proinflammatory

cytokines, such as IFN-γ, IL-18, GDF-15, and TWEAK, can destabilize plaques, while TGF-β promotes stabilization. Cytokines like IFN-γ, TNF-α, and IL-1β promote apoptosis of macrophages and foam cells, leading to enlargement of the lipid core. Additionally, these cytokines induce apoptosis of smooth muscle cells, resulting in the thinning of the fibrous cap. Moreover, pro-inflammatory cytokines inhibit the synthesis of components within the extracellular matrix involved in plaque stabilization, particularly those produced by smooth muscle cells. For instance, IFN-γ inhibits collagen synthesis by smooth muscle cells.

Macrophages infiltrate the thinned fibrous cap and release a multitude of inflammatory cytokines and proteases, including Matrix Metallo Proteinases (MMPs). These enzymes degrade the stabilizing matrix, thereby playing a crucial role in weakening and ultimately rupturing the atherosclerotic plaque. It has been reported that necrosis of the vulnerable plaque results from a combination of macrophage death and impaired phagocytic clearance of apoptotic cells. This process accelerates or triggers plaque disruption by releasing inflammatory cytokines and matrix proteases. Additionally, the mechanical stress exerted by the necrotic core on the overlying cap may contribute to plaque rupture.

For a significant duration, there was a misconception that the most severe coronary events resulted from mildly stenotic plaques. However, research on severe ST-segment Elevation MI (STEMI) cases revealed that the average constriction of the lesion lumen diameter, excluding the thrombus, exceeds 60% (Zhang *et al*., 2018). In post-sudden death investigations, 70% of ruptured plaques exhibited more than 75% vascular cross-section narrowing. Inconsistencies in studies led to the exclusion of non-small lesions in those with sequential coronary angiograms (Narula *et al*., 2013). These studies consistently reveal plaque progression as a stage between non-obstructive subclinical atherosclerosis and acute coronary events.

In the prospective study of severe coronary syndrome patients, high-risk non-culprit lesions, initially mild at angiography, doubled in size between baseline (32±21%) and the event (65±16%; $p<0.001$) (Xie *et al*., 2014). Temporary plaque increases quadrupled event likelihood. In the dynamic registry of the national heart, lung, and blood institute, average diameter stenosis increased from baseline (42±21-84±14%) during subsequent events. STEMI studies with sequential angiograms described plaque development preceding MI (Pontone *et al*., 2017). Mean stenosis diameter in lesions leading to STEMI rose from 37±21% over three months pre-event to 59±32% during STEMI. A Japanese study with successive angiograms for a year showed rapid lumen stenosis increase linked to severe coronary events in >70% of patients (Kotronias *et al*., 2021). Patients with gradual stenosis elevation in all 4 angiograms developed anginal symptoms, while those without changes had uncomplicated courses (Shin *et al*., 2015). Despite similar baseline nonobstructive disease and treatment, fast plaque development significantly increased the chance of plaque rupture and MI (Ose, 2011).

Atherothrombosis: A Complication of the Atherosclerotic Plaque

Healthy Endothelium is the Crucial Sign of Thromboresistance

The endothelial layer serves as a semi-permeable barrier, regulating the diffusion of plasma molecules, vascular tone, inflammation, and clot formation. The integrity of the endothelial barrier relies on the presence of intercellular complexes (such as occludin, claudin, connective adhesion molecules 70, cadherin, and slit compounds) and integrin receptors (Komarova *et al*., 2017; Stefanadis *et al*., 2017; Soldatov *et al*., 2018). Densely packed compounds maintain intercellular binding, influencing the growth and survival of endothelial cells, while slit compounds primarily facilitate intercellular binding, allowing the passage of water, ions, and small molecules (Castro Dias *et al*., 2019). Integrins, acting as receptors for vitronectin and fibronectin, govern the adhesion of the endothelial monolayer to the extracellular matrix.

A robust endothelium without atherosclerotic lesions exhibits high resistance to thrombosis, preventing the formation of blood clots and the occurrence of ischemic events (Gimbrone Jr and García-Cardeña, 2016).

The endothelial layer, in reality, expresses a diverse array of molecules possessing antiplatelet, anticoagulant, and fibrinolytic properties.

Platelets, vital for preventing bleeding, play a key role in clot formation on damaged blood vessel walls. These small, nucleus-free cells circulate in the bloodstream, adhering to dysfunctional areas on the vessel lining when it's damaged. This adhesion is crucial for blood clot formation, especially under conditions like high blood shear rates. Platelet receptors interact with von Willebrand Factor (vWF) and collagen, activating platelets to form a hemostatic plug essential for wound healing. In summary, platelets contribute significantly to the prevention of excessive bleeding by creating clots at damaged sites in blood vessels.

TF's Significance in Atherothrombosis

Open TF-inducing thrombin and further fibrin monolayer generation covering the area of open vascular damage is the initial trigger in atherosclerotic plaques. Subsequently, thrombosis develops with platelet dominance, which is rapidly activated and recruited into a developing thrombus (Brouns *et al*., 2020).

HIF-1α, an oxygen-sensitive transcription factor, crucially responds to local hypoxia by activating the transcription of genes like VEGF, fibroblast growth factor, cytokines, and Angiopoietins (Angs). Silencing HIF-1α in macrophages reduces proinflammatory factor production and increases macrophage apoptosis.

On the other hand, the absence of HIF-1α in antigen-presenting cells leads to polarization towards Th1 response and worsens atherosclerosis by promoting the production of inflammatory cytokines.

In endothelial cells, the transcription factor Forkhead box p (Foxp1) has been recognized as a crucial regulator that suppresses the expression of inflammasome components such as NLRP3, caspase 1, and IL-1β.

Foxp1 modulation in endothelial cells influences atherosclerosis progression, as confirmed by Zhang *et al*. (2018) study in transgenic mice.

Interestingly, researchers have found that Foxp1 is regulated by Krüppel-like factor 2 (Klf2) and both proteins are diminished in regions of blood vessels that are prone to atherosclerosis due to disturbed blood flow.

The extracellular TF domain triggers a coagulation cascade in flowing blood. Found in foam cells and lipid-enriched vascular smooth muscle cells, TF interacts with plasma Factor (F) VII/VIIa. The TF: FVIIa complex activates FIX and FX, leading to the conversion of prothrombin into thrombin. Thrombin then transforms fibrinogen into fibrin and activates factor XIII, enhancing fibrin cross-linking and stabilizing the thrombus with platelets. In this phase, a nonocclusive coronary thrombus may cause angina, or a thrombus fragment might detach, leading to micro-infarctions (distal embolization) as it blocks smaller vessels.

There is interesting evidence that C-Reactive Protein (CRP) demonstrates thrombotic activity (Kunutsor *et al*., 2017). Therefore, it was previously established that the monomeric c-reactive protein form has a certain importance in platelet adhesion.

Circulating c-reactive protein in its native (pentameric) form doesn't affect platelet deposition (Boncler *et al*., 2019). However, the monomeric form exhibits a prothrombotic phenotype, initiating platelet deposition and thrombus progression. Monomeric c-reactive protein dissociates from its pentameric form on activated platelet surfaces, facilitated by GPIIb/IIIa activation. Additionally, microparticles released during cell activation or apoptosis can convert native C-reactive protein to its monomeric form (Boncler *et al*., 2019).

Platelet recruitment is triggered by locally stored mediators upon platelet adhesion/activation, with crucial roles played by Thromboxanes A2 (TXA2) and ADP, in combination with thrombin (Braune *et al*., 2020). TXA2, generated through PLA2 stimulation, binds to TX receptors, amplifying platelet recruitment and activation. ADP, released from dense granules, increases platelet aggregation via P2Y1 and P2Y12 receptors, launching PLC-mediated calcium increase and cAMP production suppression (Karim *et al*., 2015).

Thrombin, a central protease in blood coagulation, activates platelets through PAR-1 and 4 receptors, triggering multiple signaling pathways (Koupenova and Ravid, 2018) G-protein-coupled receptors contribute to platelet shape change, granule release, TXA2 generation, GPIIb/IIIa activation and procoagulation reactions (Duvernay *et al*., 2017). Activated platelets output phosphatidylserine, stimulating the procoagulation reaction and undergo conformational changes in the GPIIb/IIIa receptor, promoting platelet aggregation (Holinstat, 2017).

These processes involve new platelets and other circulating cells, contributing to injury. Thrombin-mediated conversion of fibrinogen into fibrin stabilizes and increases the thrombus. Acute occlusive growth of a coronary thrombus can lead to acute coronary syndromes and, in some cases, sudden coronary death (Holinstat, 2017).

Differences Between Human and Animal Atherosclerotic Plaque

The majority of animal models of atherosclerosis are based on mice, rats, rabbits, and guinea pigs. Bigger animals are less popular, but birds, swine, dogs, cats, and non-human primates are still used as model animals. However, numerous differences in the pathogenesis of atherosclerosis limit the investigations. For example, in mice, rats, dogs, and cats an induction of hypercholesterolemia or atherosclerosis is very difficult because these animals have high HDL levels, which have an anti-atherosclerotic effect. Watanabe heritable hyperlipidemic rabbits express non-functional LDL receptors, which recognize some VLDL remnants, but not LDL. The suitable choice in the scope of atherosclerosis induction is the use of genetically-modified models. Thus, mice with double-knockout of ApoE- and LDL-receptor were created. Unfortunately, being fed with a normal diet, these mice do not exhibit lesions more developed than early foam-cell, fatty-streak stage (Veseli *et al*., 2017; Mironov *et al*., 2020).

Another important difference is that in experimental animals the atheroma narrows the vascular lumen, while in humans this is not common. This can be explained by the differences between the intima in large arteries of humans and model animals. Thus, human intima includes cells of different types, as well as the intima of large animals. Such cell types include pericytes and maybe SMCs, while the intima of small animals consists of only endothelium and the basal membrane. In humans, the vast majority (84-93%) of the intimal cells exhibit antigens of smooth muscle cells and pericyte-like stellate cells (Andreeva *et al*., 1992).

Conclusion

Thrombosis carries serious risks to life and health. Despite the fact that in most cases fragmentation of atherosclerotic plaques does not lead to the formation of blood clots as such, such a problem still exists. Thrombus formation on disrupted plaques is influenced by factors such as vascular wall thrombogenicity, altered blood flow, and imbalances in blood hemostasis. Studies conducted on both human and animal models of atherothrombosis have revealed significant factors that contribute to the process of thrombus formation and propagation. These factors include platelets, extrinsic and intrinsic coagulation factors, pro-inflammatory factors, plaque hypoxia, and alterations in blood flow. In some cases, platelets concentrate around the plaque fragment and form a thrombus, which can clog the vessel and lead to gangrene or heart attack. The molecular basis of this or the fate of a fragment of an atherosclerotic plaque is not fully understood. Most likely, the reason lies in the different levels of secretion of factors that stimulate platelet aggregation. However, such a concern still exists.

Platelet activation and the discharge of granules appear to be pivotal in both the initiation of atherosclerosis and acute atherothrombosis.

Nevertheless, despite such point-like findings, the development of atherothrombosis as a complication of atherosclerosis has yet to be elucidated.

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Author's Contributions

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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