Overview of Atherosclerotic Plaque: From Formation to Complication

Anastasia Vladimirovna Poznyak, Varvara Alexandrovna Orekhova, Vasily Nikolaevich Sukhorukov, Alexandra Alexandrovna Melnichenko, Mikhail Aleksandrovich Popov and Alexander Nikolaevich Orekhov

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 platelet aggregation (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).

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.
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).

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-B1 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−/−/IL4−/− 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 rupture 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 oculudin, 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 (Koupnova 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 phosphatidyserine, 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 thombogenicity, 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.

Acknowledgment

Thank you to the publisher for their support in the publication of this research article. We are grateful for the resources and platform provided by the publisher, which have enabled us to share our findings with a wider audience. We appreciate the efforts of the editorial team in reviewing and editing our work, and we are thankful for the opportunity to contribute to the field of research through this publication.

Funding Information

This study was supported by the Russian science foundation (Grant # 23-65-10014).

Author’s Contributions

Anastasia Vladimirovna Poznyak: Drafted Written.
Varvara Alexandrovna Orekhova, Vasily Nikolaevich Sukhorukov, Alexandra Alexandrovna Melnichenko, Mikhail Aleksandrovich Popov and Alexander Nikolaevich Orekhov: Drafted reviewed and edited.

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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https://doi.org/10.1161/01.ATV.19.6.1518


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