# THE ATHEROSCLEROSIS-INFLAMMATION RELATIONSHIP – A PATHOPHYSIOLOGICAL APPROACH

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## Abstract

Atherosclerosis is the predominant pathological substrate of cardiovascular diseases and through its major complications (ischemic heart disease and stroke) represents the main cause of mortality and morbidity in the developed countries. It is a chronic inflammatory disease derived from the aggregation of lipids and different types of immune cells along with macrophages, B and T cells in the arterial wall. Recent studies revealed that during atherogenesis, the interaction between lipids and immune cells represents the driving force in the chronic inflammation of the arterial wall. The present paper compiles the pathophysiology of atherosclerotic disease as it describes the involvement of the immune system, analysing the important roles of endothelial dysfunction, macrophages, LDL oxidation and smooth muscle cell migration and proliferation.

## Rezumat

Ateroscleroza este principalul substrat patologic al bolilor cardiovasculare și prin complicațiile sale majore (boala cardiacă ischemică și accidentul vascular cerebral) reprezintă principala cauză de mortalitate și morbiditate în țările dezvoltate. Este o afecțiune inflamatorie cronică care rezultă din acumularea de lipide și mai multe tipuri de celule implicate în imunitate, incluzând macrofage, limfocite B și T la nivelul peretelui arterial. Studiile recente au arătat că, în timpul aterogenezei, interacțiunea dintre lipide și celulele imune reprezintă o forță motrice a inflamației cronice a peretelui arterial. Această lucrare analizează fiziopatologia bolii aterosclerotice, din perspectiva contribuției sistemului imunitar, punând în evidență rolul crucial al disfuncției endoteliale, macrofagelor, oxidarii LDL, migrării și proliferării celulelor musculare netede.

**Keywords**: atherosclerosis, inflammation, endothelial dysfunction

## Introduction

Cardiovascular disease (CVD) represents the most prevalent cause of death worldwide, leading to 17.3 million deaths annually, accounting for 31.5% of all deaths [35]. More than 4 million cardiovascular deaths occur in Europe annually, with higher rates among women (2.1 million) than among men (1.8 million). Ischemic heart disease (IHD) and stroke are the main causes of mortality in Europe, responsible for 1.267.000 deaths (28% of all deaths) among men and 1,460,000 deaths (33%) among women, each year [47]. In Romania the death rates pe year from all CVD are around 149.060 (58.50%), IHD and stroke being responsible for 45.041 (30.21%) deaths among men and 50.630 (33.96%) among women [51]. Studies have shown that over 19% of adults in Romania have 3 or more vascular risk factors including dyslipidaemia [7]. The prevalence of dyslipidaemia among the adult population in our country is over 46% [10].

Atherosclerosis is one of the leading risk factors underlying CVD, and the clinical manifestations are coronary artery disease (CAD), stroke, transient ischemic attack (TIA), cerebral aneurysm, vascular malformation, stenosis and occlusion of non-cerebral and non-coronary arteries and aortic aneurysm. The vascular complications of atherosclerosis are acute myocardial infarction (IM), stroke and ischemic limbs and viscera [38, 39].

It is considered that atherosclerosis is a lifelong inflammatory condition influencing the large and medium-sized arteries defined by a complex interaction between endothelial dysfunction, lipid deposits, innate and adaptive immune system reaction, smooth muscle cell (SMC) migration and proliferation and extra-cellular matrix reshaping, that can lead to the development of an intimal plaque [1, 33]. The atherosclerotic process is triggered by endothelial dysfunction which allows lipoproteins to enter and accumulate in the sub-endothelial space where they go through chemical and

physical changes making them more pro-inflammatory and activating endothelial cells (ECs) [9, 32, 55]. Activated ECs induce the expression of adhesion molecules which mediate the rolling and firm arrest of monocytes and T cells. Once entered in the intima, monocytes become macrophages, under the action of two growth factors granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage-colony-stimulating factor (MCS-F), that upregulate toll-like receptors (TLRs), and with the help of scavenger receptors (SRs) engulf oxidized LDL (oxLDL), resulting in lipid aggregation and foam cell development [15, 25].

The onset is insidious, atherosclerosis can appear early in life, with a long asymptomatic phase that can progress under the influence of cardiovascular risk factors. Different classifications of cardiovascular risk factors are in use. The traditional or conventional CV risk factors (hypertension, hypercholesterolemia, smoking, diabetes mellitus) were the first described, to which new or non-traditional risk factors (abdominal obesity, microalbuminuria, anaemia, metabolic syndrome) were added [19].

In this review, we focus on current opinions regarding the pathological mechanisms of the major factors involved in atherosclerosis, including biomechanical factors, endothelial dysfunction, LDL oxidation, with a major accent on immune system activation and SMC migration and proliferation.

**The role of biomechanical factors in atherosclerosis**

Blood, immune cells, oxygen, paracrine hormones, are delivered in every cardiac cycle through arteries and arterioles under pulsatile pressure to peripheral organs. Complex biomechanical forces are generated on the vessel wall as the result of the interactions between pulsatile blood flow and arterial geometries.

The arterial wall, endothelial cells, and atherosclerotic tissue are deformed as a result of these complex mechanical forces. These forces act in circumferential, radial and axial directions and induce stress into the vessel wall [8, 45]. Wall (or endothelial) shear stress (WSS) and plaque structural stress (PSS), are the two most important biomechanical factors that have been hypothesized to be implicated in the induction and evolution of atherosclerosis.

WSS represents the parallel force vector component applied by the blood flow onto the vascular wall, stretching the artery in the longitudinal direction. Its tension increases proportionally with blood velocity and viscosity, so with increased blood flow, WSS also intensifies, decreasing the vascular tone of SMC and therefore produces vasodilation. Shear stress is the stress located either into the normal arterial wall (WSS) or within the atherosclerotic plaque (PSS), as a result of the vessel extension and stretch induced by arterial pressure [2].
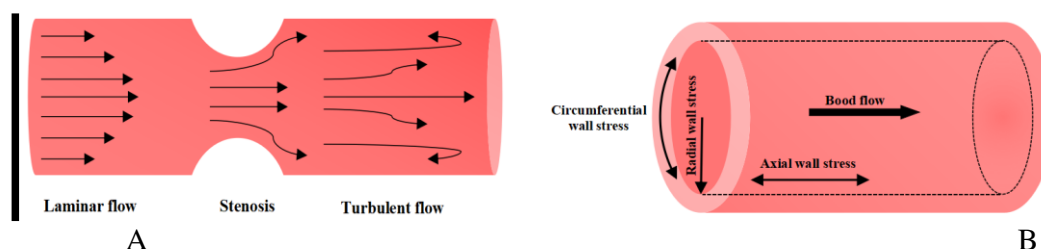


**Figure 1.**
The influence of stenosis and hemodynamic forces on the blood flow
(A). Atherosclerotic plaque causes narrowing of the vascular wall resulting in blood flow changes, making the transition from laminar flow to turbulent flow; (B). Hemodynamic forces acting on the blood vessel wall are: the circumferential wall stress; axial wall stress and radial wall stress

The atherosclerotic plaque causes narrowing of the arterial lumen resulting in increased blood velocity and therefore increased shear stress within the stenotic region. Additionally, the endothelium next to the narrowed area is exposed to WSS.

Short-term changes of shear stress lead to functional changes in the arteries. Prolonged intensified hemodynamic stress leads to the initiation of endothelial lesions. As a consequence of the increase in shear stress, activated ECs secrete prostacyclin and nitric oxide (NO), which promote vasodilation [6, 17].

**Endothelial dysfunction in atherosclerosis**

Vascular homeostasis is mainly regulated by endothelial cells and one of its most important functions is to maintain the vasoconstriction-vasodilation balance, thrombogenesis and fibrinolysis, stimulation and prevention of SMC proliferation and transfer into the intimal layer [21, 22]. When this is disrupted, a disturbance of the endothelial function occurs, inducing an impairment in the arterial wall.

Healthy endothelium undergoes vascular repair and remodelling, senses and mechanotransduces hemodynamic forces. NO initially identified as an endothelium-derived relaxing factor (EDRF), is the main vasodilatory substance released by ECs. Prostacyclin and bradykinin are generated by cyclooxygenase (COX) in the endothelium and also exhibit vasodilatory properties [36, 37]. Prostacyclin has significant antiplatelet effects and thus limits platelet aggregation [21, 22]. Bradykinin

induces the deliverance of NO, prostacyclin, and endothelium-derived hyperpolarizing factor, that is an important vasodilator in the microcirculation, promoting the inhibition of the platelet aggregation process [36, 37]. Bradykinin is essential for fibrinolysis by stimulating tissue plasminogen activator (t-PA). Endothelin (the most effective endogenous vasoconstrictor described to date) and angiotensin II are two vaso-constricting substances, also produced by ECs that contribute to plaque development by stimulating the proliferation of smooth muscle cells [37, 54].

The imbalances between vasoconstriction and vaso-dilation in response to endothelial dysfunction, lead to maladaptive responses that stimulate or increase atherosclerosis; these involve increased endothelial permeability, platelet aggregation, leukocyte adhesion and cytokines release [54]. One of the first signs of atherosclerosis may be a reduced release or activity of NO, manifested as disturbed vasodilation. NO is generated *via* an oxidation reaction of L-arginine in the presence of endothelial NO synthase (eNOS), produce L-citrulline and stoichiometric amounts of NO. Tetra-hydrobiopterin (THB4) and nicotinamide adenine dinucleotide phosphate (NADPH) are 2 cofactors involved in NO production [30].

Once generated, NO diffuses into SMC, activating intracellular guanylate cyclase, increases cyclic guanosine monophosphate (cGMP) levels, decreasing intra-cellular calcium levels and promoting vasodilation [24].

## The role of oxLDL

High amounts of circulating lipids have an important role in the progression of atherosclerosis. Apolipo-protein B (ApoB) is the principal structural apolipo-protein in low-density lipoproteins (LDL), vital for its construction and growth. ApoB exists in 2 forms in the human body: ApoB100 which is present in the liver and therefore it appears on the liver-derived lipo-proteins very-low-density-lipoprotein (VLDL), intermediate density lipoprotein (IDL) and LDL [11]. Another form is ApoB48 that is present in the intestine and it is expressed on chylomicrons. During atherosclerotic lesion development, LDL represents the main source of lipid accumulation within the arterial wall. *In vitro* studies have shown that intracellular cholesterol accumulation is induced by atherogenic modified LDL and not by native LDL. Modified LDL is internalized especially through unregulated phagocytosis following a different metabolic pathway compared to native LDL [49].
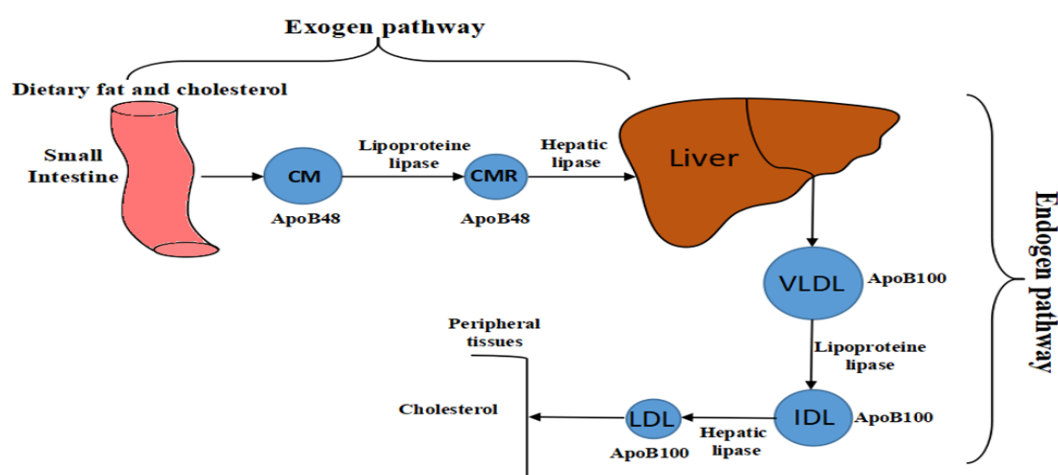


**Figure 2.**

Lipid metabolism. CM, chylomicrons; CMR, chylomicrons remnant; Apo B48, apolipoprotein B48; VLVL, very low-density lipoprotein; IDL, intermediate density lipoprotein; LDL, low-density lipoprotein; Only the predominant lipids are shown

Through transcytosis, lipoproteins with a diameter smaller than 70 nm have the capacity to transverse the endothelium, where ApoB containing lipoproteins bind to proteoglycans (such as heparan, chondroitin, dermatan and keratin) through electrostatic binding [14, 31, 48]. Subsequently binding to proteoglycans, LDL undergoes a physical change concerning ApoB100 and lipid composition [4, 23, 28]. In patients with atherosclerosis, plasma analysis revealed different types of LDL alterations including the acquisition of negative electric charges, glycation, desialylation, and acetylation [34]. Proteoglycan-bound LDL thus becomes

more prone to oxidation, generating inflammation and ECs activation. Once traped at the subendothelial level, LDL oxidation occurs in the presence of reactive oxygen and nitrogen species released by vascular cells [5, 42,].

## Immune system activation in atherosclerosis

It was thought that atherosclerotic plaques develop passively by deposition of lipids in the endothelium. However, it was shown that atherosclerosis is the result of chronic inflammation within the arterial wall caused

by the accumulation of LDL and leukocytes. The pathophysiology of atherosclerosis is complex and it is believed to start with lipid accumulation at the level of the intima, tissue macrophage activation and production of cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). Cytokines stimulate endothelial production of adhesion molecules such as E- and P-selectins, vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) [20]. These molecules facilitate endothelial adhesion and transmigration into the intima of various leukocytes (monocytes, T and B-cells, neutrophils). Monocytes present several membrane structures capable of interacting with adhesion molecules such as P-selectin glycoprotein ligand-1 (PSGL-1) or monocyte-expressed very late antigen-4 (VLA-4) [43].
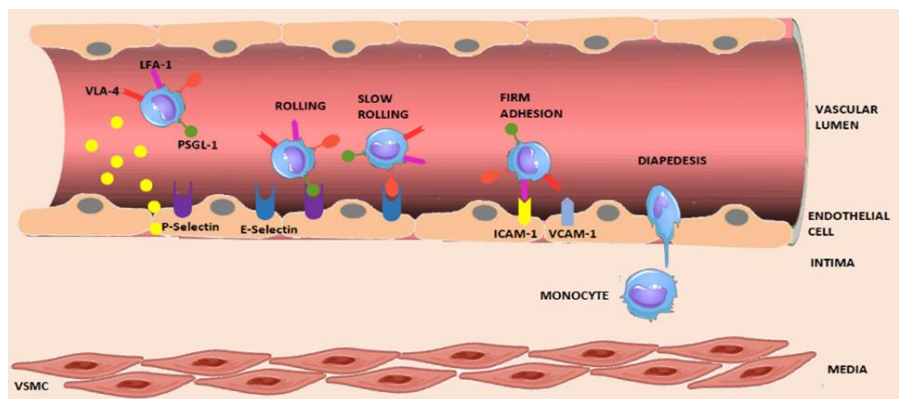


**Figure 3.**
Monocytes endothelial adhesion and transmigration into the intima

Monocytes are activated to macrophages by different growth factors such as monocyte-colony stimulating factor (M-CSF), as well as cytokines such as TNF-α, IL-1β, interleukin-4 (IL-4), interleukin-8 (IL-8), inter-leukin-13 (IL-13) and interferon-γ (IFN-γ). Atherosclerosis involves the transformation of monocytes into macrophages. M-CSF-knockout mice display resistance to atherosclerosis development [39]. Subsequently, IFN-γ triggers macrophages to produce neopterin, a potentially useful atherosclerosis and inflammation marker [39].

Recent studies have shown that cytokines may influence macrophage activation [29]. In this respect, two major types of macrophages have been identified: M1 and M2. M1 secretes pro-inflammatory cytokines such as IL-12, IL-6 and TNF-α and may enhance plaque vulnerability. M2 produces anti-inflammatory cyto-kines such as IL-10 and TGF-β and increases plaque stability. M1 differentiation is stimulated by IL-1β and IFN-γ secreted by T-helper cells 1 (Th1), whereas M2 differentiation is stimulated by IL-4 and IL-13 secreted by Th2 [29].

Reactive oxygen species (ROS) are formed under various conditions such as myeloperoxidases release by neutrophils or IFN-γ release by macrophages and other cells. ROS oxidize LDL molecules at different degrees. In the first stage, LDL oxidation produces a minimally oxidized LDL (mmLDL), with pro-inflammatory activity. Further oxidation of mmLDL leads to moderately oxidized LDL (moLDL) formation, and finally, even further oxidation leads to strongly oxidized LDL (oxLDL). Macrophages express scavenger receptors: SR-A1, Toll-like receptor 4 (TLR-4), lectin-type oxidized LDL receptor 1 (LOX-1) or CD36 and CD68 that can distinguish between mmLDL, moLDL and oxLDL [44]. TLR-4 recognize mmLDL, whereas LOX1, SR-A1 and CD36 identify moLDL and oxLDL [44]. Upon expression of scavenger receptors, macro-phages uptake and phagocyte oxLDL, thus becoming foam cells.

Dendritic cells (DCs) are important components of the immune system, which have recently been found to be involved in atherosclerosis. There are four major types of DCs: conventional, plasmacytoid, monocyte-derived and Langerhans cells. Granulocyte macrophage colony stimulating factor (GM-CSF) or TLR-4 ligands influence the transformation of monocytes into monocyte-derived DCs. Monocyte-derived DCs generate proinflammatory cytokines (TNF-α, IL-6 and IL-12), but also atheroprotective cytokines such as IL-10. DCs express scavenger receptors (LOX-1, CD36 and CD205) which facilitate oxLDL uptake and the activation of intracellular pathways leading to enhanced pro-inflammatory cytokines production [39].

Atherosclerotic lesions present T cells in a lesser extent compared to macrophages. Activation of T cells is the result of the interactions with antigens, leading to pro-inflammatory cytokines production and worsening of atherosclerosis. Most T cells involved in athero-sclerosis are Th1. They secrete IFN-γ which activates macrophages and recruits vascular smooth muscle cells to inhibit collagen synthesis, thus destabilizing the plaque. IFN-γ is thought to be an interesting therapeutic target in atherosclerosis. Th2 cells have

controversial roles in atherosclerosis. They decrease IFN-γ production but also stimulate antibody production by B cells, and secrete cytokines like IL-4, IL-5, IL-9 and IL-13. Regulatory T cells (Tregs) are important inflammation suppressors by producing IL-10 and by regulating other adaptive immune responses. Other T cells involved in pro-atheroslerogenesis are: Th17, CD4 + T cells and CD8 + T cells, although Th17 has also displayed atheroprotective effects [27].

B cells can be identified in atherosclerotic plaques in a lesser extent compared to T cells. Their roles in atherosclerosis are controversial. The use of an antibody against CD20 has resulted in the inhibition of atherosclerosis progression. Also, studies show that anti-oxLDL IgM, have atheroprotective effects, whereas oxLDL-specific IgG have atherosclerotic effects [39].

In conclusion, atherosclerosis development and progression involve many components of both innate and adaptive immune systems. Currently, there is an increasing interest in the study of the relationship immune system-atherosclerosis for diagnostic and prognostic purposes, but also for therapeutic targeting in atherosclerosis [13, 16, 52].

**Smooth muscle cells migration and proliferation**

The main support for the vessel wall structure is provided by smooth muscle cells (SMC), by vascular tone regulation and thus maintenance of blood pressure and perfusion. SMC present increased plasticity in order to accomplish various functions: extracellular matrix synthesis, contraction and proliferation [46].

SMC can present with two phenotypes: the contractile phenotype, which mediates vasodilatation and vaso-constriction, and the proliferating phenotype [12]. As a reaction to injury, the contractile phenotype will transform into the proliferative phenotype.

Morphological and functional properties of SMC may change under the influence of growth factors, inflammation mediators and mitogens. SMC may be unable to contract, but gain abilities such as migration, proliferation and accumulation in the intima [40]. In response to injury, macrophages secrete various growth factors such as: platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin growth factor (IGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and transformer growth factor (TGF) and also angiotensin II (AngII).

The PDGF induces the migration of smooth muscle cells from media to intima. IL-1, thrombin and TNF-α stimulate PDGF activity [26]. Once the SMC migrate, factors such as FGF, heparin affinity growth factor (HB-EGF) and PDGF stimulate cell growth and division [50]. Also, VEGF contributes to SMC proliferation [26]. SMC produce the elements that form the extracellular matrix. This matrix consists of type I collagen and type III collagen, elastin and proteoglycans. Extracellular matrix formation is controlled by matrix metallo-proteinases (MMPs), which due to their enzymatic activity destroy various macromolecules within the matrix [3].

SMC can lead to vascular remodelling and intimal vascular lesions if they fail to shift to the contractile phenotype [18].

The proliferating phenotype of SMC diminishes contractile protein expression and increases the expression of extracellular matrix proteins, thus controlling vascular remodelling [41]. SMC accumulation in the intima is an important process in advanced atherosclerotic lesions. This accumulation is followed by slow proliferation, with various intensities of cell division due to intermittent rupture in the atheromatous plaque. Cell proliferation is inhibited by various cytokines (TGF-α, IFN-γ) [53]. Additionally, SMC apoptosis inhibits proliferation, which explains the increased fibrous content and reduced cellular architecture in the mature atheromatous plaque.
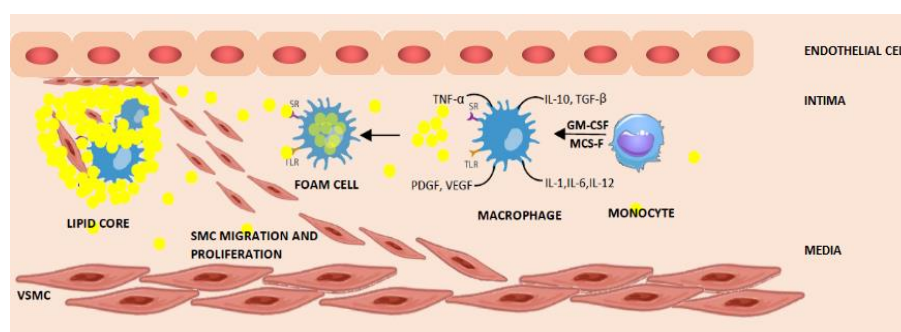


**Figure 4.**
Smooth muscle cell migration and proliferation. Once entered in the subendothelial space, monocytes differentiate into macrophage, under the influence of two growth factors: GM-CSF and M-CSF. Macrophages, through scavenger receptors (SR) and Toll-like receptors (TLR) engulf the oxLDL, releasing pro-inflammatory cytokines (TNF-α, IL-1, IL-6, IL-12) and become foam cells. Activated macrophages also release platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), two factors that induce the migration of smooth muscle cells from the media to the intima

It must be mentioned that SMC that migrate to the intima are morphologically different from native SMC from media. Under the action of external stimuli, SMC change morphologically by reducing the number of monofilaments and increasing organelles involved in protein synthesis (the Golgi apparatus and the endoplasmic reticulum) [12]. Thus, SMC found in the atheromatous plaque are histologically immature but have increased secretory activity.

Similar to macrophages, SMC can accumulate lipo-proteins at the cytoplasmic level, transforming into foam cells.

## Conclusions

Atherosclerosis is a multifactorial disease whose pathogenesis is not fully understood, but inflammation and its complex cellular processes play an important role in the initiation and progression of athero-sclerotic lesions, therefore, administration of compounds with anti-inflammatory potential could be a valuable therapeutic solution.

Immuno-inflammatory mechanisms are directly involved in the initiation and progression of cardiovascular diseases from the incipient asymptomatic stage of vascular injury to clinical manifestations of dysfunction and vascular remodelling.

Given the multifactorial nature of this process, additional studies are essential for improving our knowledge in this field, from experimental models to human tissue assessment, systemic biomarkers, epidemiological and clinical observations.

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