

Maya Simionescu and Anca V. Sima

2.1 Atherosclerosis Is a More Complex Process than Previously Thought

Atherosclerosis is a multifactorial and multipart progressive disease manifested by the focal development within the arterial wall of lesions – the atherosclerotic plaques – in response to various deleterious insults that affect the vessel wall's cells. Among the risk factors, as identified by classical epidemiology, there are dyslipidemia, vasoconstrictor hormones incriminated in hypertension, products of glycoxidation associated with hyperglycemia, pro-inflammatory cytokines and smoking, out of which the first is a prerequisite for the initiation and progression of about half of arterial lesions. In other instances, an inflammatory reaction induced by putative antigens that stimulate T lymphocytes, certain heat shock proteins, components of plasma lipoproteins, and potentially, microbial structures induce atherosclerotic plaque in the absence of systemic hypercholesterolemia [1, 2]. Thus, the process is more complex than previously thought. The conventional view that stressed the role of dyslipidemia in the generation of atherosclerosis was rounded by extensive evidence that inflammation is a key contributor to all stages of this disease, from the initial lesion to the ruptured plaque [2]. In all cases, the atheroma formation entails a progressive process in which the gradual implication of various cells and their secretory products define a sequence of events that leads from the fatty streak to fibro-lipid plaque, and ultimately to plaque rupture and atherothrombosis.

M. Simionescu (✉) • A.V. Sima
Institute of Cellular Biology and Pathology “N. Simionescu” of the Romanian Academy, 8 B.P.
Hasdeu Street, 35-14050568 Bucharest, Romania
e-mail: maya.simionescu@icbp.ro

2.2 Vascular Resident Cells and Circulating Blood Cells Emigrated Within the Arterial Wall Participate to Atherosclerotic Plaque Formation

Tunica intima, media and adventitia are the three layers that make up the vessel wall. The intima is made essentially of a monolayer of epithelial-like cells, the endothelial cells (EC), resting on a basal lamina they produce, and a few smooth muscle cells (in human arteries) that also synthesize their own basal lamina and contribute to the extracellular matrix (ECM) proteins. The internal elastic lamina separates the intima from the media that comprises numerous layers of smooth muscle cells (SMC), bordered by a basal lamina and intercalated between elastic laminae, all embedded within the ECM. An external elastic lamina separates the media from the adventitia, which is made up mainly of fibroblasts, mast cells, microvessels, lymphatic vessels and nerves, housed within an extended ECM.

The resident cells of the arterial wall (EC and SMC) in concert with cells emigrated from the blood (in particular T-lymphocytes, monocytes, dendritic cells, mast cells) and their secretory products (chemokines, cytokines, enzymes), through ample cross talk and signalling, contribute to the initiation, evolution and fate of the atherosclerotic plaque.

2.3 Atheroma Formation Is a Progressive Process Arbitrarily Delineated by Consecutive Stages

In the last 30 years, thorough exploration of the cellular and molecular modifications occurring in the arterial areas susceptible to plaque development uncovered the sequence of events and the consecutive stages that take place within the continuous process of atherosclerosis, either in hypercholesterolemic conditions or as an inflammatory reaction, in the absence of hypercholesterolemia (Fig. 2.1). Within this continuous process a pre-lesional, often reversible, phase (Fig. 2.1, stages I, II and III), occurring during the first three decades of life, and a phase of progressive atherosclerotic plaque formation (Fig. 2.1, stages IV, V and VI) can be distinguished.

2.3.1 Stage I. Commencement of Plaque Formation: Modulation of Endothelial Constitutive Functions

By position and large surface area exposed to the blood, EC are the first cells to experience the impact of any minute perturbation occurring in the blood or interstitial fluid homeostasis. In arterial lesion-prone areas, the initial event that takes place in response to changes in body homeostasis (i.e. hyperlipidemia, hyperglycemia, inflammation) is the modulation of EC constitutive functions.

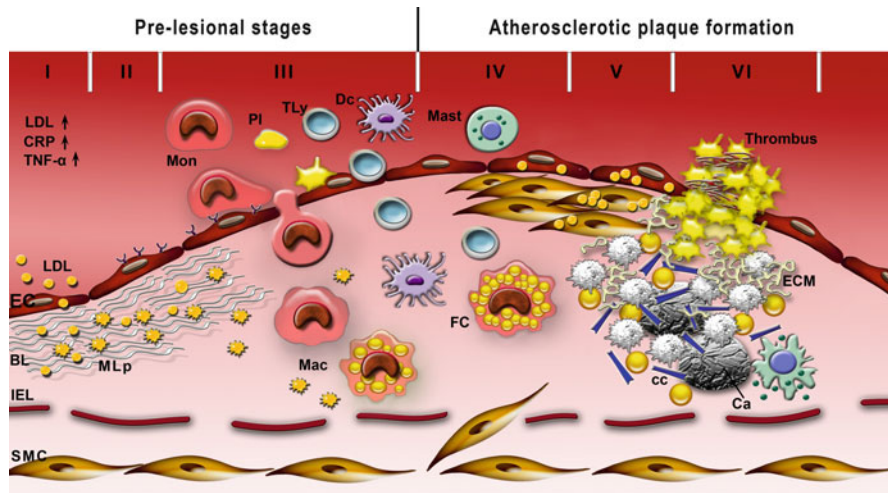


Fig. 2.1 Diagrammatic representation of the atheroma formation and the arbitrarily delineated consecutive stages. **Stage I**, the initiation of the plaque, is characterized by modulation of endothelial cell (*EC*) constitutive functions due to increased concentrations of plasma low density lipoproteins (*LDL*), or C-reactive Protein (*CRP*) and tumor necrosis factor (*TNF- α*). In **stage II**, *LDL* trapped within the intima undergo alterations (oxidation, glycation), turning into modified lipoproteins (*MLp*). They induce *EC* dysfunction, expressed by the appearance of new cell adhesion molecules and chemotactic factors. This is followed by a robust inflammatory reaction (**stage III**) in which plasma monocytes (*Mon*) assisted by platelets (*Pl*), T lymphocytes (*TLy*) and dendritic cells (*Dc*) adhere and enter the arterial intima. Monocytes become activated macrophages (*Mac*) that express scavenger receptors, take up *MLp* and progressively turn into macrophage derived-foam cells (*FC*) characteristic for the fatty streak. In **stage IV**, smooth muscle cells (*SMC*) glide from the media into the intima forming the fibrous cap. The fibrolipid plaque comprising *SMC*-, *Mac*- and *EC*-derived foam cells defines **stage V**. Extracellular matrix (*ECM*), cholesterol crystals (*cc*) and large calcification cores (*Ca*) are formed. The complicated plaque becomes vulnerable, exhibiting fibrous cap thinning and excess inflammatory cytokines that leads to **stage VI**, characterized by *EC* damage and death and the subsequent exposure of the matrix, platelets adherence and thrombus formation (*Thrombus*), *BL* basal lamina, *ECM* extracellular matrix

- (a) Modification of *EC* controlled permeability and the ensuing increased transcytosis and deposition of plasma *LDL* within the intima

The close positive correlation between aortic *LDL* permeability in a given segment and the cholesterol accumulation in that particular segment suggests that the aortic permeability to *LDL* is a predictor for the development of cholesterol-induced experimental atheroma formation [3].

In experimental atherosclerosis models, plasma *LDL* concentration gradient generates a prominent increase in transcytosis [4–6]. The latter, in conjunction with the reduced efflux of *LDL* predominantly to the lumen of the artery [7] and the subsequent trapping of *LDL* within the subendothelial matrix concur to their accumulation in the subendothelium, within and outside the basal lamina, against the fragmented internal elastic lamina (Fig. 2.2).

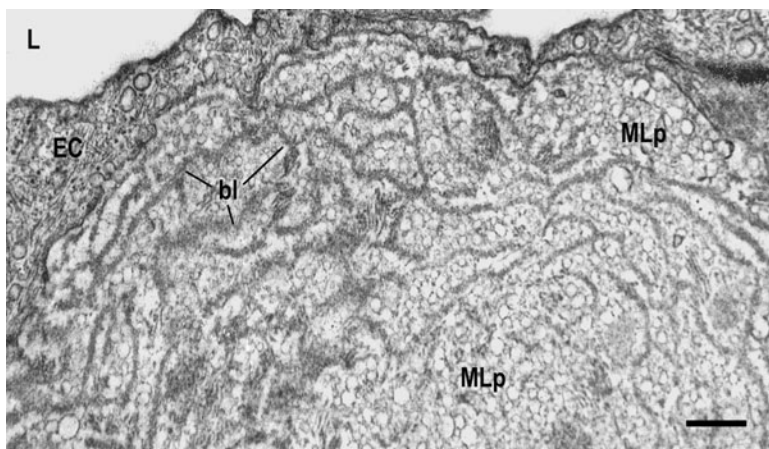


Fig. 2.2 Trapping of modified lipoproteins (*MLp*) in a hyperlipidemic hamster vessel's intima. Note the subendothelial accumulation of *MLp* within the hyperplastic, multilayered basal lamina (*bl*). *EC* endothelial cells, *L* vascular lumen. Bar: 1 μ m

The intima-confined LDL interact with proteoglycans and matrix proteins that, among other factors, trigger their atherogenic conversion into oxidatively modified Lp (*MLp*), as demonstrated in animal models [8–11] and human aorta [12, 13]. The *MLp* are heterogeneous structures and appear in situ or after their isolation from experimental animals or human aorta as vesiculated, aggregated, or fused particles rich in unesterified cholesterol [12, 13]. Accumulation and retention of Lp in the subendothelium [14, 15] depend both on EC and Lp characteristics, such as their oxidation susceptibility [16]. The atherogenic modifications of LDL may take place either within the plasma, or when crossing the EC or within the subendothelial ECM. It is possible that the alteration of LDL occurs to different degrees in all three locations [17]. The small fraction of altered LDL (oxidised, glycated, enzymatically-modified, etc.) detected in circulation is possibly due to the powerful antioxidant systems existing in the plasma, as well as to the scavenger receptors (i.e. for asialoglycoproteins) present in the liver and other organs of the mononuclear phagocytic system.

LDL has been consistently confirmed as a major risk factor for cardiovascular diseases (CVD) and is the basis of statins treatment. However, lipoproteins (Lp) alone do not explain all of the risks inherent in CVD; one-half of all heart attacks and strokes occur among individuals without hypercholesterolemia, and one-fifth of all cardiovascular events occur in the absence of any of the major risk factors. C-reactive protein is a circulating pentraxin that plays a major role in human innate immune response and provides a stable plasma biomarker for low-grade systemic inflammation. Among patients with stable angina and established CVD, plasma levels of CRP have consistently been associated with recurrent risk of cardiovascular events [18].

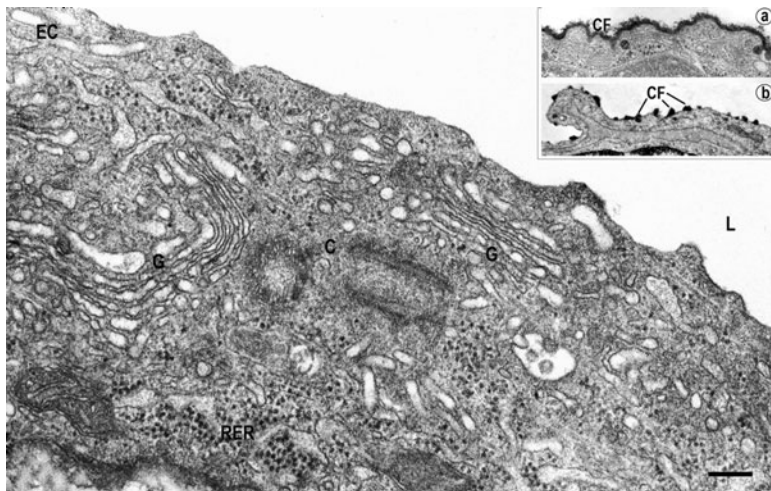


Fig. 2.3 Structural modulation of endothelial cell (*EC*) denoting a secretory phenotype occurring in the initial stage of atherogenesis. Note the presence of numerous copies of rough endoplasmic reticulum (*RER*), Golgi complexes (*G*) and centrioles (*C*) in the aortic valve of a hyperlipidemic hamster. Bar = 200 nm. *Inset*: (a) cationic ferritin (*CF*) decorates uniformly the *EC* plasmalemma of a normal hamster aorta. (b) In hyperlipidemic hamsters, diminution and clustering of anionic sites on the aortic *EC* plasmalemma occurs. *L* vascular lumen

(b) Changes in *EC* phenotype

Almost concurrently with increased transcytosis and retention of MLp within the intima, major changes in the biosynthetic capacity of *EC* occur. Structurally, *EC* switch to a secretory phenotype characterized by multiple copies of the rough endoplasmic reticulum, Golgi apparatus, centrioles and numerous caveolae (Fig. 2.3). Functionally, a progressive development of a hyperplastic multilayered basal lamina entrapping steadily MLp within its meshes takes place [19–21] (Fig. 2.2). The proliferation of the basal lamina and of the ECM disrupt the myo-endothelial junctions, as well as the gap junctions between neighbouring SMC, leading to an altered response of the vessel wall to external stimuli.

(c) Alteration of the endothelial net negative surface charge

Under normal conditions, the *EC* plasmalemma has a net negative charge that contributes to the characteristic non-thrombogenic surface of the endothelium (all circulating cells expose also a negatively charged surface) (Fig. 2.3, inset). In experimental atherosclerosis, the arterial *EC* plasmalemma exhibits gradually a reduced and non-homogenous distribution of anionic sites (revealed with *in situ* perfused cationic ferritin), as opposed to the uniform decoration of the *EC* membrane in control animals [22]. Long-term hyperlipidemia results in redistribution of anionic sites, which are significantly reduced on the *EC* plasmalemma, whereas they become clustered on the diaphragms of the caveolae (Fig. 2.3, inset). One can safely assume that the diminished arterial endothelial surface negative charge may account, in part, for the increased

permeability and the augmented adhesive characteristics of the vessel wall in specific arterial locations [23].

2.3.2 Stage II. EC Dysfunction

The dual assault on EC luminal and abluminal surface, namely the alteration of plasma lipid homeostasis and the subendothelial accrual of MLp generates, as a defence reaction, the initiation of a multipart inflammatory process manifested at first by the EC “activation”; this general term designates a set of stimuli-generated dysfunctions that elicit new structural and functional properties.

In early human and experimental atherosclerosis, the alterations of the EC non-adhesive and non-thrombogenic surface are an illustration of EC dysfunction. The latter is manifested by the expression on the EC plasmalemma of new or additional cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), E-selectin, and P-selectin (which function in the selective recruitment of monocytes), fractalkine, vascular cell adhesion molecule (VCAM)-1 that binds to monocyte cognate receptors (VLA-4 and CCR2) and trigger their adherence to EC. In addition, the cells synthesize monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8), which are chemoattractant for monocytes [24], a trio of CXC chemokines that function in T-lymphocytes recruitment [1] and eotaxin, which is chemoattractant for mast cells (via CCR3 receptors); all these molecules are overexpressed in human atherosclerotic plaque [25]. This set of molecular and cellular changes, denoting EC activation and dysfunction, is a defence reaction assisting the vascular endothelium to recruit specifically blood inflammatory cells.

2.3.3 Stage III. Robust Inflammatory Reaction: Adhesion and Extravasation of Monocytes and Lymphocytes, Fatty Streak Formation

- (a) Adhesion, diapedesis and residence within the intima of a specific set of pro-inflammatory *monocytes*

Blood monocytes are heterogeneous; the two major human monocytes subsets are the $CD14^+CD16^-$ and $CD14^-CD16^+$ [26]. The pro-inflammatory monocytes (characterised by specific surface molecules) undergo a coordinated process in which monocyte integrins and EC adhesion molecules and chemokines orchestrate the capture, rolling, adhesion and arrest of monocytes on the endothelium lining the MLp-rich arterial segments. The adhesion process is followed by transmigration (diapedesis) of monocytes through the EC junctions and their residence within the subendothelium (Fig. 2.4) [27, 28].

- (b) *Platelets* assist the recruitment of blood monocytes, thus participating to the initiation of plaque formation

Initially considered the main contributor to arterial thrombosis, platelets were recently revealed as participants to all stages of atherosclerosis. In the early

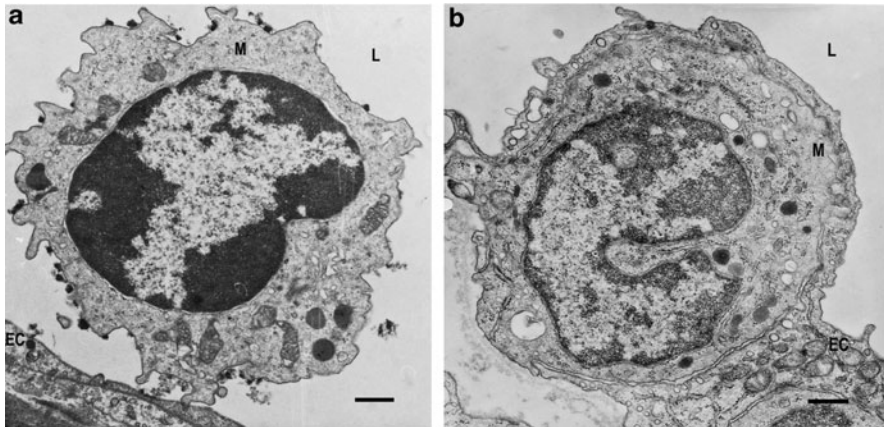


Fig. 2.4 (a) Adherence to an endothelial cell (*EC*) and (b) subendothelial residence of blood monocytes in a hyperlipidemic hamster aorta. *L* vascular lumen. Bar: 500 nm

phase, activated EC secrete and expose on their surface von Willebrand factor (vWf). The latter, by interacting with platelets glycoprotein Ib, triggers the recruitment and adherence of platelets to the intact EC surface. Upon adhesion, platelets are activated and secrete a variety of pro-inflammatory cytokines and chemoattractants (platelet factor 4, RANTES, P-selectin, soluble CD40 ligand, MMPs). The platelet membrane P-selectin mediates EC-platelet interaction also. The interaction between platelet P-selectin with monocyte P-selectin glycoprotein ligand-1 [29] leads to the formation of platelet–monocyte aggregates (Fig. 2.5); the activated platelets promote leukocyte binding to the vascular cell adhesion molecule-1 (VCAM-1) and increased their adhesiveness to inflamed or atherosclerotic endothelium. In atherosclerotic carotid arteries platelet–leukocyte aggregates are often detected; individual platelets directly adherent on atherosclerotic endothelium are rarely found [30]

(c) *Recruitment of T-Lymphocytes*

T-cells, mostly $CD4^+$ T cells, are recruited and present in the prelesional stages of atherosclerotic plaque formation together with antigen-presenting dendritic cells and some $CD8^+$ T cells [1, 31]. Circulating T-cells migrate into the atherosclerotic lesions in response to chemokines, monokines and chemoattractants, which bind to the cell specific receptors (Fig. 2.6). Within the plaque various antigens, such as MLp, induce T-cell proliferation (reviewed in [32]). Upon recognition of an antigen, the type 1 helper T cells (Th1) become activated, express and secrete a large array of cytokines and cell surface molecules, which contribute to macrophage activation and the potentiation of the inflammatory response.

(d) *Dendritic cells* are required for the activation of naïve T cells

Dendritic cells are specialized antigen presenting cells that are required for the activation of naïve T cells and the development of antigen-specific T cell

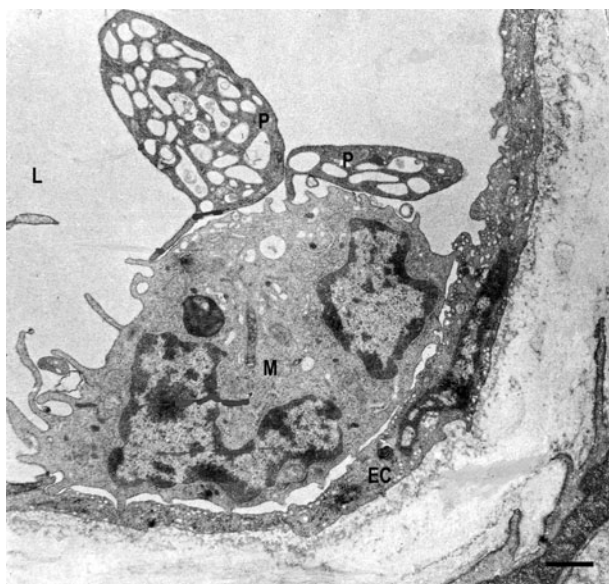


Fig. 2.5 The interaction between endothelial cells (*EC*), a circulating cell, most likely a monocyte (*M*) or a neutrophil, and activated platelets (*P*) in an early stage of aortic plaque formation in a hyperlipemic hamster. *L* vascular lumen. Bar: 0.8 μ m

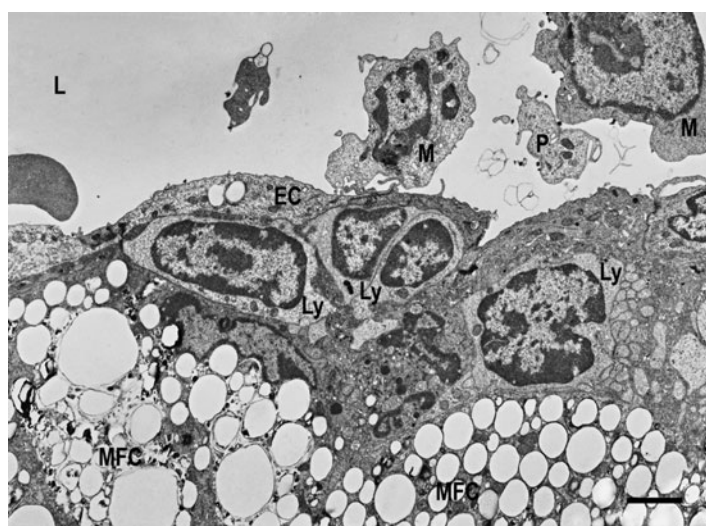


Fig. 2.6 Endothelial cells (*EC*), a group of lymphocytes (*Ly*) and macrophage-derived foam cells (*MFC*), are in close contact in a developing atheroma. Note, within the aortic lumen (*L*), the close contact between a monocyte (*M*) and an EC containing lipid droplets, and between a platelet (*P*) and a circulating monocyte. Bar: 1.7 μ m

mediated immune responses. They are present from the early stages till the advanced atherosclerotic plaque, particularly in the rupture-prone shoulder region of the lesions [33]. MLp and other stimuli that accelerate atherogenesis (i.e. TNF- α) augment dendritic cells adhesion to the endothelium and their subsequent transmigration [34]. Vascular dendritic cells either in normal or in atherosclerotic arterial intima appear as a network of elongated cells with extended long, dendritic-like processes, often found in contact with T cells in areas of neovascularization and in the adventitia vasa vasorum (reviewed in [35]). It is possible that in these areas (as in other parts of the immune system) the vascular dendritic cells present antigens to naïve T cells, since these cells retain the antigen presenting function under conditions typical for atherosclerotic plaques [36].

- (e) *Polymorphonuclear neutrophils (PMN)*, whose role in inflammation is well established, were for a long time considered of insignificant relevance in atherosclerosis. Accumulated indirect evidence in humans and animal models indicate a close relationship between the number of circulating activated PMN, the coronary artery disease and their presence into culprit lesions [37]. Activated PMN release superoxide and pro-inflammatory mediators at the blood – vessel wall interface that may affect the EC properties, promote or amplify the recruitment of inflammatory cells and within the plaque, by the molecules they secrete, may contribute to its vulnerability [38]. Moreover, recent evidence indicates that in hypercholesterolemia-induced neutrophilia, PMN infiltrate arteries primarily during early stages of atherosclerosis, suggesting their role in the initiation of atherosclerosis. Moreover, lesion progression in Apoe^{-/-} mice was blunted by depletion of circulating PMN, indicating a significant impact of PMN on atherosclerosis in this murine model [39].

The adhesion molecules that mediate rolling, adhesion and transmigration of PMN are present in atherosclerotic lesions. The paucity of PMN found in atherosclerotic lesion could be due to either their rapid apoptosis within the plaque [40], or to the cytotoxic effect of free fatty acids released from modified Lp [41]. In addition, there are indications that PMN may be important during destabilization of advanced plaques [42].

More research is necessary to acknowledge the mechanisms by which PMN contribute to atherogenesis and its progression. Thus far, the data imply a causative role of PMN in the inflammatory conditions associated with atherogenesis and athero-progression and suggest the potential importance of modulating neutrophilic inflammation as part of the strategy to prevent/treat atherosclerosis (reviewed in [43]).

- (f) *Mast Cells*, known for their role in allergy, have recently being acknowledged as pro-inflammatory effector cells present in the human arterial intima and in evolving atherosclerotic lesions. When activated, mast cells secrete the rich content of their cytoplasmic granules, such as histamine, neutral proteases, growth factors, and pro-inflammatory cytokines within the plaque. These factors act on MLp, extracellular matrix, and intimal cells neighbouring the activated mast cells. Moreover, the immunoglobulin G immune complexes

containing MLp, present within the human atherosclerotic lesions, activate mast cells inducing the secretion of numerous pro-inflammatory cytokines (TNF- α , IL-8 and MCP-1) and the release of histamine and tryptase [44]. Thus, mast cells may contribute to fatty streak formation and to the generation of unstable plaques susceptible to rupture (reviewed in [45]).

- (g) *B cells* were initially found within the vessels' adventitia, and later immunoglobulin-positive cells were detected within the atherosclerotic plaques. Recently, indirect evidence on experimental animals revealed that *B cells* direct the immune response during the development of the atherosclerotic plaque and their immunoglobulin products may perform protective functions during the plaque progression (reviewed in [35]).
- (h) Intimal differentiation of monocytes into activated macrophages and the subsequent formation of macrophage-derived foam cells

Within the intima, monocytes differentiate into macrophages by a regulated program that includes the upregulation of scavenger receptors (i.e. SR-B1 and CD-36). Scavenger receptors are operational in the uptake of MLp, advanced glycosylation endproducts, anionic phospholipids and even apoptotic cells. The non-regulated uptake of MLp mediates the switch of activated macrophages into cholesterol loaded macrophage-derived foam cells. Accumulation of macrophage-derived foam cells is the hallmark of fatty-streak type lesion (Fig. 2.7), which ultimately may evolve to advanced fibro-lipid plaque (reviewed in [46]).

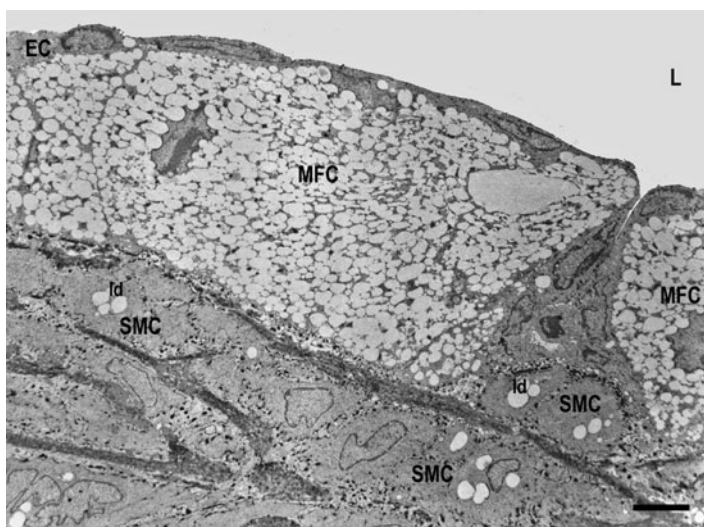


Fig. 2.7 Fatty streak lesion in a hyperlipidemic hamster aorta. Numerous macrophage-derived foam cells (MFC) amass under a continuous, thin endothelium. A few smooth muscle cells (SMC) emigrated from or within the media contain lipid droplets (*ld*). EC endothelial cells, L vascular lumen. Bar: 2 μ m

2.3.4 Stage IV. Fibrous Plaque Formation

While some human lesions may begin as intimal xanthomas, there is considerable evidence suggesting that the intimal thickening is most likely precursor leading to symptomatic coronary disease, since these lesions are found in children in similar locations as advanced plaques in adults, while fatty streaks are known to regress [47].

The inflammatory cells, through the factors they secrete within the plaque, send molecular messages: macrophage-derived-foam cells secrete cytokines, growth factors, tissue factor, IFN-gamma, matrix metalloproteases (MMPs), and produce reactive oxygen species (ROS); lymphocytes secrete among others CD-40 L. These messages govern the plaque development, including the clonal accumulation of SMC within the intima (reviewed in [21]).

A crucial event in the formation of the plaque is the migration of SMC from the vessels media into the intima, through the fragmented, partially degraded internal elastic lamina. The areas known as “intimal thickenings” can be either “eccentric” or “diffuse”, although these two types are often contiguous and can be difficult to distinguish from each other. Eccentric intimal thickenings tend to be focal and involve up to half of the circumference of the arterial wall. They are found in conserved locations, including branchpoints and areas of turbulent blood flow [47]. Other sources for intimal SMC, besides those migrated from the media, are the circulating bone marrow cells and the vascular progenitor cells present in the adventitia of all arteries. Just like the activated EC, the migrated SMC switch to a secretory phenotype, resulting in a hyperplastic, multilayered basal lamina and enlarged extracellular matrix enriched especially in collagen bundles and fibrils. The conversion of quiescent SMC and EC to a secretory phenotype may represent a functional adaptation/modulation of these cells to protect themselves from the vicious microenvironment [21]. In the coronary arteries of hyperlipemic hamsters, intimal SMC proliferate, accumulate lipid droplets, and further turn into foam cells [23] contributing to the formation of fibro-lipid lesions in the affected arteries (Fig. 2.8).

2.3.5 Stage V. Calcified Atherosclerotic Fibro-Lipid Plaque

The advanced atherosclerotic plaques are characterized by accumulation of extracellular lipid droplets, macrophage-, and SMC-derived foam cells, and calcification cores that develop further into big calcification centres, which occupy a large part of the coronary artery in experimental hyperlipemic hamsters (Fig. 2.9) and humans [20, 48].

Accumulation of free cholesterol within the plaque is a potent inducer of apoptosis of macrophage derived-foam cells; in addition, within the lesions, SMC and T-cells may also go through apoptotic cell death [46]. Apoptotic cells release their content initiating the formation of the necrotic core. The defining feature of this stage is a lipid rich necrotic core encapsulated by fibrous tissue [49]. Excess extracellular unesterified cholesterol nucleates into cytotoxic crystals and the

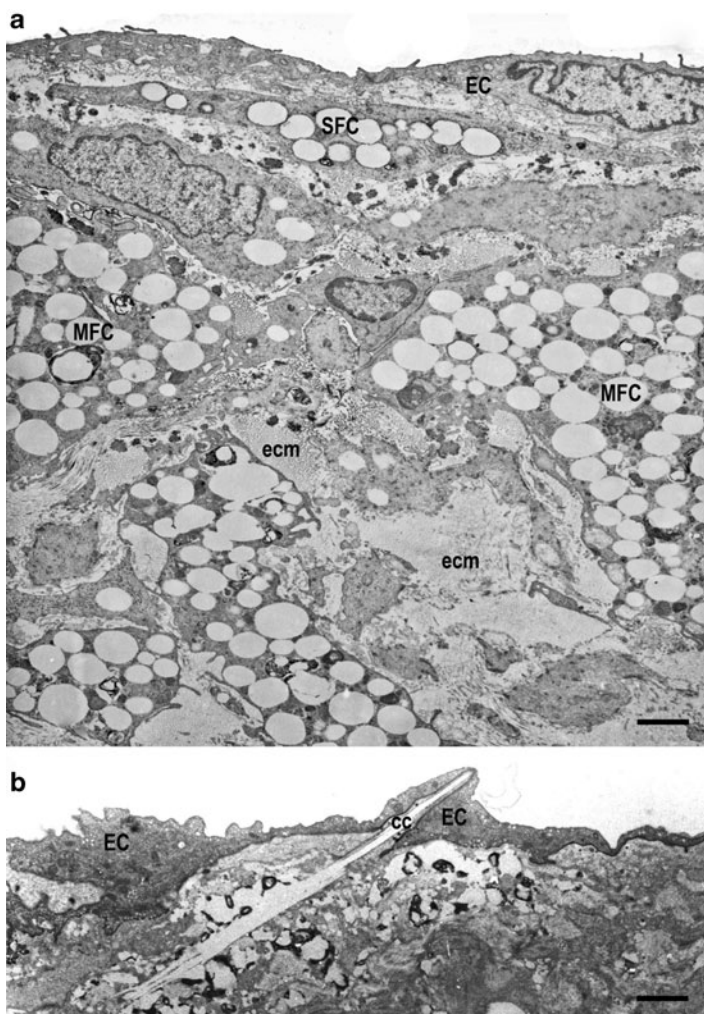


Fig. 2.8 Fibrolipid plaque located in a hyperlipemic hamster aorta. **(a)** The endothelium (*EC*) overlays numerous macrophage-derived foam cells (*MFC*) and smooth muscle-derived foam cells (*SFC*). Note the expanded extracellular matrix (*ECM*) and the scattered fragments of elastic lamellae. Bar: 1 μm . **(b)** A large cholesterol monohydrate crystal (*cc*) originating from the extracellular lipid deposits, aggressing an endothelial cell (*EC*) and almost penetrating through the cell. Bar: 1.3 μm

atherosclerotic plaque evolves to complicated atheroma, eventually causing the total occlusion of coronary artery branches [20].

The fibro-lipid lesions endowed with a robust fibrous cap are considered stable plaques (Fig. 2.10a). Thinning of the fibrous cap concomitant with its infiltration with macrophages and T-lymphocytes, cellular apoptosis, and the accumulation of large cholesterol crystals generates the unstable (vulnerable) plaque (Fig. 2.10b),

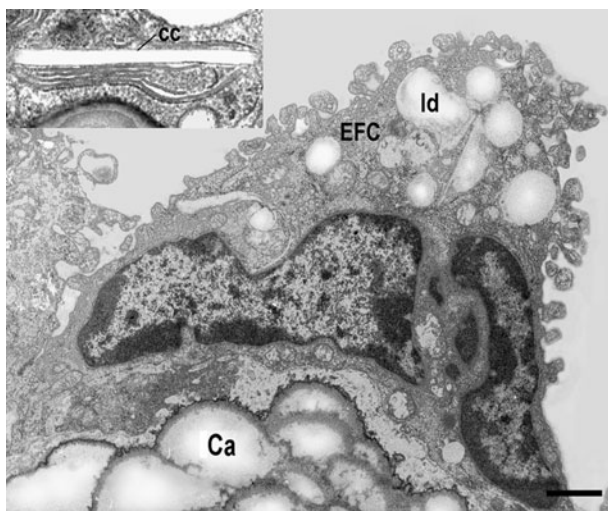


Fig. 2.9 Endothelial-derived foam cell (*EFC*) in a late stage of experimental hypercholesterolemia. The cell exhibits numerous membrane blebs, cytoplasmic lipid droplets (*ld*) and sometimes intracellular cholesterol crystals (*cc*, *inset*). Note the presence of a considerable calcium deposit (*Ca*) in the subendothelium. Bar: 400 nm

which is prone to rupture and the ensuing thrombus formation, that can occlude the lumen and cause myocardial infarction or stroke [50].

2.3.6 Stage VI. Complicated Plaque: Rupture, Thrombosis

The exact mechanism of plaque rupture is not known, but it includes cap thinning, excess inflammatory cytokines and proteases that mediate digestion of the matrix, decreased collagen synthesis and the presence of injured or apoptotic cells within the necrotic core. All the cells that contribute to the formation of the atherosclerotic plaque are also implicated in the plaque rupture and the consequent thrombosis.

Endothelial cells covering the fibrous cap become either extremely thin (Fig. 2.10b) or loaded with lipid droplets turning into EC-derived foam cells (Fig. 2.9); in either case, they are fragile and susceptible to erosion. Ultimately, the EC are injured and their disruption exposes the ECM (rich in pro-inflammatory and pro-coagulant molecules) to the circulating blood cells, that initiate the thrombus formation.

Macrophages infiltrate the thinned fibrous cap. They express and secrete a large number of inflammatory cytokines and proteases, especially MMPs, which digest the stabilizing matrix, thus having a key role in the weakening and ultimate rupture of the atherosclerotic plaque. Necrosis of the vulnerable plaque is due to a combination of macrophages death and defective phagocytic clearance of apoptotic cells. The dead or dying macrophages release an excess of inflammatory cytokines and

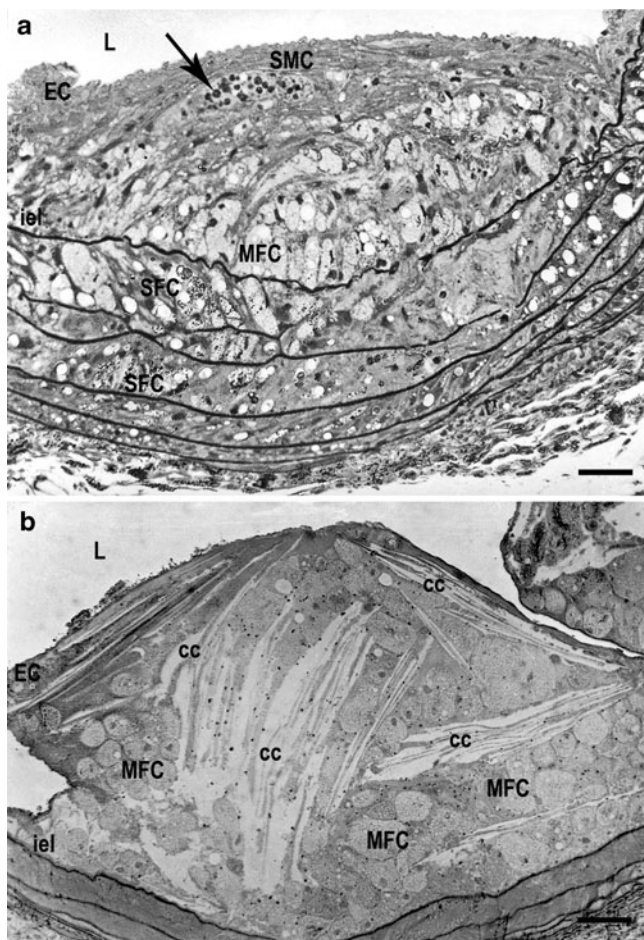


Fig. 2.10 Stable and vulnerable atherosclerotic plaques. (a) A stable fibrolipid plaque, exhibiting numerous macrophage-derived foam cells (*MFC*) under a well developed fibrous cap comprising smooth muscle cells (*SMC*) and infiltrated leukocytes (*arrow*); in between elastic laminae there are SMC-derived foam cells (*SFC*). (b) Vulnerable plaque exhibiting a large number of live and apoptotic *MFC*, massive accumulation of oversized cholesterol crystals (*cc*), under a thinned, but continuous layer of endothelial cells (*EC*). *L* vascular lumen, *iel* elastic laminae. Bar: 4 μm

matrix proteases that accelerate and/or induce plaque disruption. The mechanical stress caused by the necrotic core to the overlying cap may also produce the plaque rupture (reviewed in [49]).

SMC also participate to cap thinning. They exhibit a decrease in collagen synthesis that concurrently with the proteolytic digestion of the extracellular matrix by MMPs cause the thinning of the fibrous cap and the resulting rupture of the plaque.

Mast cells that are located especially in the rupture-prone shoulder regions of the plaque, secrete proteases (tryptase and chymase) that assist the destabilization of the atherosclerotic plaque [35].

Platelets have a major role in the thromboembolic complications of the vulnerable plaque. When the plaque ruptures, platelets adhere to the exposed extracellular matrix rich in pro-inflammatory factors, become activated, aggregate and form a thrombus on the surface of the disrupted lesion. The overlying thrombus is often in continuity with the underlying necrotic core rich in macrophages [51]. Thrombotic vascular occlusion is associated with ischemic episodes, such as acute coronary syndrome or cerebral infarction. Acute thrombosis is predominantly characterized by layered platelet aggregates with variable amounts of fibrin, red blood cells and acute inflammatory cells. At least 75–80% of sudden coronary deaths show occlusive acute or organized thrombi. In some cases, the matrix heals by a concerted biological process involving the infiltration of SMC, accumulation of extracellular matrix proteins (i.e., proteoglycans and collagen), neovascularization, inflammation, and luminal surface re-endothelialization [52].

2.4 Adventitia in Atherosclerosis

The role of the outermost layer of blood vessels, the adventitia (from the Latin “adventicius” meaning extraneous) in atherosclerosis was to a certain extent neglected; however, lately, the implications of this connective tissue-rich layer, which houses besides fibroblasts, microvessels that constitute the vasa vasorum, lymphatic vessels, mast cells, nerves and progenitor cells have been revealed.

The structural components of the adventitia undergo significant changes with the advancement of the atherosclerotic plaque formation. In humans, with the development of the lesions, the adventitial layer becomes infiltrated with inflammatory cells, initially with macrophages and T-lymphocytes and ultimately (in advanced stages) with B-lymphocytes [53].

Vasa vasorum is the source of neo-vessels that sprout into the atherosclerotic plaque. They are the provider of oxygen and nutrients for the cells present within the hypoxic microenvironment of the atheroma, thus contributing to its development. In humans, plaque neovascularization takes place in the early phases of atherosclerosis and is associated with the inflammatory reaction. The thin-walled new microvessels lined by a discontinuous endothelial layer and lacking SMC are also a conduit and a source of more inflammatory cells, monocytes/macrophages, T cells and mast cells, within the atherosclerotic plaque. Disruption of newly formed microvessels leads to intra-plaque hemorrhage and the ensuing accumulation of erythrocytes that may be a consequence or may induce plaque instability in the advanced atherosclerotic lesions (reviewed in [54]). Together, accumulated data highlight a key role for the adventitia in the development of atherosclerosis.

2.5 Conclusion

The atherosclerotic plaque is the end product of the activity and reactivity of the resident cells of the arterial wall, which under the assault of a large variety of aggressors and risk factors elicit an inflammatory process, manifested by the attraction and implication of the cells of the immune system.

EC are the first cells to react to minute changes occurring in the microenvironment and the last to surrender (i.e. cell death). They go progressively through modulation of their constitutive function (permeability, biosynthesis), followed by dysfunction (expression of new cell adhesion molecules and chemoattractants) and ultimately, in late stages of atherosclerosis, to injury and death. The EC dysfunction initiates a robust inflammatory reaction and the atherogenic MLP sustain and propagate the inflammatory response. The cross talk and the messages exchanged among the arterial wall cells depend on the mediators of inflammation and immunity.

SMC migrated in the intima from the tunica media, proliferate and elaborate a rich and complex extracellular matrix. They form the fibrous cap that stabilizes the plaque, and in concert with EC and macrophages, secrete MMPs that modulate numerous functions of vascular cells, including proliferation, migration, as well as neoangiogenesis or degradation of the matrix.

As the lesion progresses, calcification and cell death commonly occurs; together with the accumulated extracellular lipids, they form the classic lipid-rich necrotic core that ultimately may rupture to generate atherothrombosis.

All the cells (resident or emigrated), every single chemokine, cytokine, MMP and vasa vasorum that contribute to the atherosclerotic plaque formation are the bona fide targets for therapeutic intervention to stop or retard its progression. There are already in use suitable drugs for lowering LDL blood concentration, the statins. Further pharmacological advances will allow reaching targets beyond LDL, such as increasing HDL level, blocking plaque neovascularization, or employing stem cells for regenerative strategies. Expectations in the field are dependent on the accumulated knowledge on the intricate cellular and molecular processes of atherosclerosis and on treating the cell as the “basic patient”. The future hope for patient-tailored treatment implies concurrently a “cell-tailored treatment”.

Acknowledgements The authors are grateful to all the scientists who have made great contributions to the field of atherosclerosis, to our eminent mentor, Professor Nicolae Simionescu, and our great collaborators who, over the years, have contributed to the data presented in this review. We thank Mrs. Marilena Daju for the excellent graphical design and image processing.

The support of the Romanian Academy, the Romanian Ministry of Education and Research grant #1027/2008 and European COST Action BM0602 is gratefully acknowledged.

References

1. Millonig G, Malcom GT, Wick G (2002) Early inflammatory-immunological lesions in juvenile atherosclerosis from the pathobiological determinants of atherosclerosis in youth (PDAY)-study. *Atherosclerosis* 160:441–448
2. Libby P, Ridker PM, Göran K (2009) Hansson, inflammation in atherosclerosis, from pathophysiology to practice. *J Am Coll Cardiol* 54:2129–2138
3. Nielsen LB, Nordestgaard BG, Stender S, Kjeldsen K (1992) Aortic permeability to LDL as a predictor of aortic cholesterol accumulation in cholesterol-fed rabbits. *Arterioscler Thromb* 12:1402–1409
4. Nordestgaard BG, Hjelm E, Stender S, Kjeldsen K (1990) Different efflux pathways for high and low density lipoproteins from porcine aortic intima. *Arterioscler Thromb Vasc Biol* 10:477–485
5. Schwenke DC, StClair RW (1993) Influx, efflux, and accumulation of LDL in normal arterial areas and atherosclerotic lesions of white Carneau pigeons with naturally occurring and cholesterol aggravated aortic atherosclerosis. *Arterioscler Thromb* 13:1368–1381
6. Vasile E, Antohe F, Simionescu M, Simionescu N (1989) Transport pathways of beta-VLDL by aortic endothelium of normal and hypercholesterolemic rabbits. *Atherosclerosis* 75:195–210
7. Nordestgaard BG, Hjelm E, Stender S, Kjeldsen K (1990) Different efflux pathways for high and low density lipoproteins from porcine aortic intima. *Arteriosclerosis* 10:477–485
8. Faggiotto A, Ross R, Harker L (1984) Studies of hypercholesterolemia in the nonhuman primate I. Changes that lead to fatty streak formation. *Arterioscler Thromb Vasc Biol* 4:323–340
9. Kruth HS (1983) Filipin-positive, oil red O-negative particles in atherosclerotic lesions induced by cholesterol feeding. *Lab Invest* 50:87–93
10. Nistor A, Bulla A, Filip DA, Radu A (1987) The hyperlipidemic hamster as a model of experimental atherosclerosis. *Atherosclerosis* 68:159–173
11. Simionescu N, Vasile E, Lupu F, Popescu G, Simionescu M (1986) Prelesional events in atherogenesis: accumulation of extracellular cholesterol rich liposomes in the arterial intima and cardiac valves of hyperlipidemic rabbits. *Am J Pathol* 123:85–101
12. Kruth HS (1984) Localization of unesterified cholesterol in human atherosclerotic lesions. Demonstration of filipin positive, Oil-Red-O-negative particles. *Am J Pathol* 114:201–208
13. Tirziu D, Dobrian A, Tasca C, Simionescu M, Simionescu N (1995) Intimal thickenings of human aortas contain modified reassembled lipoproteins. *Atherosclerosis* 112:101–114
14. Sima A, Stancu C, Simionescu M (2009) Vascular endothelium in atherosclerosis. *Cell Tissue Res* 335:191–203
15. Tabas I, Williams KJ, Borén J (2007) Macrophages, apoptotic cells and cholesterol – strategies for survival. *Circulation* 116:1832–1844
16. Steinberg D (2009) The LDL modification hypothesis of atherogenesis: an update. *J Lipid Res* 50:S376–S381
17. Ishigakia Y, Okaa Y, Katagiri H (2009) Circulating oxidized LDL: a biomarker and a pathogenic factor. *Curr Opin Lipidol* 20:363–369
18. Tsimikas S, Willerson JT, Ridker PM (2006) C-reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. *J Am Coll Cardiol* 47:C19–C31
19. Filip DA, Nistor A, Bulla A, Radu A, Simionescu M (1987) Cellular events in the development of the valvular atherosclerotic lesions induced by experimental atherosclerosis. *Atherosclerosis* 67:199–214
20. Sima A, Bulla A, Simionescu N (1990) Experimental obstructive coronary atherosclerosis in the hyperlipidemic hamster. *J Submicrosc Cytol Pathol* 22:1–6
21. Simionescu M (2007) Implications of early structural-functional changes in the endothelium for vascular disease. *Arterioscler Thromb Vasc Biol* 27:266–274
22. Mompeo B, Popov D, Sima A, Constantinescu E, Simionescu M (1998) Diabetes induced structural changes of venous and arterial endothelium and smooth muscle cells. *J Submicrosc Cytol Pathol* 30:475–484

23. Sima A, Stancu C, Constantinescu E (2004) The hyperlipidemic hamster, a model to study atheroma formation and anti-atherosclerotic drugs effects. In: Simionescu M, Sima A, Popov D (eds) *Cellular dysfunction in atherosclerosis and diabetes – reports from bench to bedside*. Academy Publishing House, Bucharest, pp 309–322
24. Boisvert WA, Santiago R, Curtiss LK, Terkeltaub RA (1998) A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. *J Clin Invest* 101:353–363
25. Haley KJ, Lilly CM, Yang JH, Feng Y, Kennedy SP, Turi TG, Thompson JF, Sukhova GH, Libby P, Lee RT (2000) Overexpression of eotaxin and the CCR3 receptor in human atherosclerosis: using genomic technology to identify a potential novel pathway of vascular inflammation. *Circulation* 102:2185–2189
26. Geissmann F, Jung S, Littman DR (2003) Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 19(1):71–82
27. Weber C, Zernecke A, Libby P (2008) The multifaceted contributions of leukocyte subsets to atherosclerosis: lessons from mouse models. *Nat Rev Immunol* 8:802–815
28. Woollard KJ, Geissmann F (2010) Monocytes in atherosclerosis: subsets and functions. *Nat Rev Cardiol* 7:77–86
29. Davi G, Patrono C (2007) Platelet activation and atherothrombosis. *New Engl J Med* 357: 2482–2494
30. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, Littman DR, Weber C, Ley K (2003) Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med* 9:61–67
31. Hansson GK (2005) Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 352:1685–1695
32. Packard RR, Lichtman AH, Libby P (2009) Innate and adaptive immunity in atherosclerosis. *Semin Immunopathol* 31(1):5–22
33. Bobryshev YV (2005) Dendritic cells in atherosclerosis: current status of the problem and clinical relevance. *Eur Heart J* 26:1700–1704
34. Weis M, Schlichting CL, Engleman EG, Cooke JP (2002) Endothelial determinants of dendritic cell adhesion and migration: new implications for vascular diseases. *Arterioscler Thromb Vasc Biol* 22:1817–1823
35. Galkina E, Ley K (2009) Immune and inflammatory mechanisms of atherosclerosis. *Annu Rev Immunol* 27:165–197
36. Packard RR, Maganto-Garcia E, Gotsman I, Tabas I, Libby P, Lichtman AH (2008) CD11c (+) dendritic cells maintain antigen processing, presentation capabilities, and CD4(+) T-cell priming efficacy under hypercholesterolemic conditions associated with atherosclerosis. *Circ Res* 103:965–973
37. Soehnlein O, Zernecke A, Eriksson EE, Rothfuchs AG, Pham CT, Herwald H, Bidzhekov K, Rottenberg ME, Weber C, Lindbom L (2008) Neutrophil secretion products pave the way for inflammatory monocytes. *Blood* 112:1461–1471
38. Soehnlein O, Weber C, Lindbom L (2009) Neutrophil granule proteins tune monocytic cell function. *Trends Immunol* 30:538–546
39. Soehnlein O, Lindbom L, Weber C (2009) Mechanisms underlying neutrophil-mediated monocyte recruitment. *Blood* 114:4613–4623
40. Zernecke A, Bot I, Djalali-Talab Y et al (2008) Protective role of CXC receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. *Circ Res* 102:209–217
41. Luxa CA, Koschinskib A, Derscha K, Husmann M, Bhakdia S (2009) Hypersusceptibility of neutrophil granulocytes towards lethal action of free fatty acids contained in enzyme-modified atherogenic low density lipoprotein. *Atherosclerosis* 207:116–122
42. Drechsler M, Megens RTA, van Zandvoort M, Weber C, Soehnlein O (2010) Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. *Circulation* 122:1837–1845
43. Baetta R, Corsini A (2010) Role of polymorphonuclear neutrophils in atherosclerosis: current state and future perspectives. *Atherosclerosis* 210:1–13

44. Lappalainen J, Lindstedt KA, Oksjoki R, Kovanen PT (2011) OxLDL-IgG immune complexes induce expression and secretion of proatherogenic cytokines by cultured human mast cells. *Atherosclerosis* 214(2):357–363
45. Kovanen PT (2009) Mast cells in atherogenesis: actions and reactions. *Curr Atheroscler Rep* 11:214–219
46. Tiwari RL, Singh V, Barthwal MK (2008) Macrophages: an elusive yet emerging therapeutic target of atherosclerosis. *Med Res Rev* 28:483–544
47. Velican C, Velican D (1985) Study of coronary intimal thickening. *Atherosclerosis* 56: 331–344
48. Stary HC (2000) Natural history and histological classification of atherosclerotic lesions: an update. *Arterioscler Thromb Vasc Biol* 20:1177–1178
49. Tabas I, Tall A, Accili D (2010) The impact of macrophage insulin resistance on advanced atherosclerotic plaque progression. *Circ Res* 106:58–67
50. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM (2000) Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 20:1262–1275
51. Rudd JFH, Narula J, Strauss HW, Virmani R, Machac J, Klimas M et al (2010) Imaging atherosclerotic plaque inflammation by fluorodeoxyglucose with positron emission tomography. Ready for prime time? *J Am Coll Cardiol* 55:2527–2535
52. Kolodgie FD, Nakazawa G, Sangiorgi G, Ladich E, Burke AP, Virmani R (2007) Pathology of atherosclerosis and stenting. *Neuroimaging Clin N Am* 17(3):285–310
53. Watanabe M, Sangawa A, Sasaki Y, Yamashita M, Tanaka-Shintani M, Shintaku M, Ishikawa Y (2007) Distribution of inflammatory cells in adventitia changed with advancing atherosclerosis of human coronary artery. *J Atheroscler Thromb* 14:325–331
54. Langheinrich AC, Kampschulte M, Buch T, Bohle RM (2007) Vasa vasorum and atherosclerosis. Quid novi? *Thromb Haemost* 97:873–879



<http://www.springer.com/978-3-7091-0337-1>

Inflammation and Atherosclerosis

Wick, G.; Grundtman, C. (Eds.)

2012, X, 634 p., Hardcover

ISBN: 978-3-7091-0337-1