2 Atherogenesis and Vascular Biology

Peter P. Toth

Atherogenesis

Introduction

Atherosclerotic disease is highly prevalent throughout the world and is the principal cause of morbidity and mortality in Western nations for both men and women [1]. Atherosclerosis is pathophysiologically complex and begins at an early age (fatty streaks can be found in children and adolescents), with anatomically apparent coronary disease frequently becoming apparent in the third decade of life though it tends to remain clinically silent until the sixth or seventh decade [2]. Atherogenesis is driven by highly evolved networks of histologic, rheologic, autoimmune, oxidative, inflammatory, and thrombotic responses to vascular injury. These networks engage in extensive cross talk. Once established, the rate of disease progression is influenced by numerous risk factors, including age, multiple forms of dyslipidemia, hypertension, sympathetic tone, cigarette smoking, obesity, sedentary lifestyle, chronic kidney disease, the intensity of underlying inflammation, depression, insulin resistance, diabetes mellitus, urbanization, air pollution, and perhaps some forms of infection [3, 4].

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In the past six decades, an enormous range of scientific, epidemiologic, and clinical research has shown that the control of modifiable risk factors through lifestyle adjustments and pharmacologic therapies slows or even reverses the trajectory of atherosclerosis [5, 6]. Statin treatment is known to improve endothelial function, reduce inflammation, stabilize established atherosclerotic plaque, and reduce risk for such complications as myocardial infarction (MI), transient ischemic attack and stroke, claudication and peripheral arterial disease, cardiovascular and all-cause mortality, and the need for revascularization via angioplasty/stenting or bypass grafting [7, 8]. Early identification and treatment of risk factors are tantamount to the long-term prevention of atherosclerotic disease given the fact that the number of risk factors, their severity, and the duration of exposure determine lifetime risk [9–11]. Consequently, evaluating global cardiovascular risk burden, quantifying 10-year or lifetime risk, and treating each identified risk factor (e.g., dyslipidemia, hypertension, diabetes mellitus) to current guideline targets are of the essence before the onset of such clinical signs and symptoms as angina pectoris or claudication [12]. Unfortunately, little progress has been made in primordial prevention as guideline writing bodies are hesitant to make recommendations for treating adolescents and young adults [13, 14]. In a very real way, we typically wait until patients develop coronary or peripheral vascular disease

M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_2

before risk factors are identified and treated. Risk factor goal attainment rates even in patients with advanced, unstable disease tend to be distressingly low [15–18]. There continues to be considerable clinical inertia in the treatment of risk factors. *Undertreatment of risk factors does not constitute appropriate or adequate treatment of risk factors,* especially if the therapeutic goal is the prevention of disease.

The arterial system is not a simple tubular conduit network. Arteries are histologically and biochemically complex, dynamic structures that are highly responsive to their milieu. They are endowed with a wide variety of receptors along the endothelium and smooth muscle cells that regulate vasomotor tone (i.e., the capacity to regulate vasoconstriction and vasorelaxation as demanded by physiological circumstances). The coronary and cerebral vasculature are tightly regulated and fine-tuned to the oxygen delivery needs of the myocardium and the brain via local pressor effects (nitric oxide, prostacyclin, endothelin-1) as well as sympathetic and parasympathetic inputs. The coronary and peripheral vasculature (cerebrovasculature and lower extremity arteries) are continually exposed to multiple atherogenic stimuli that act additively to potentiate the pathophysiology underlying atherogenesis in the majority of persons. Atherosclerosis is a diffuse disease that, when left untreated, tends to progress throughout life.

Arterial Structure

Arteries are highly evolved, responsive conduit vessels for blood and, one of its most important constituents, oxygen. Oxygen must be available to aerobic cells in order to function as a terminal electron acceptor for mitochondrial oxidative phosphorylation. During embryological vasculogenesis, the arterial wall differentiates into three layers with distinct cellular and connective tissue constituents: these are the intima, media, and adventitia. The intima is composed of (1) an endothelial cell monolayer that interfaces with blood and (2) the lamina propria which contains smooth muscle cells, fibroblasts, collagen, and intercellular matrix that comprised glycosaminoglycans (hyaluronate, heparin/heparan sulfate) [19]. The media is composed of smooth muscle cells which regulate arterial tone and blood pressure by either contracting or relaxing in response to a variety of vasoactive molecules (e.g., nitric oxide, catecholamines, prostacyclin, bradykinin, endothelin-1, angiotensin II). The media is separated from the intima and adventitia by the internal and external elastic membranes, respectively. During atherogenesis, smooth muscle cells in the media can undergo activation via platelet-derived growth factor or cell surface lipoprotein binding proteins, rearrange their actin cytoskeleton, extend pseudopodia, and migrate into the intima where they are incorporated into atheromatous plaques [20]. The smooth muscle cell is able to migrate by releasing proteases into its surroundings which hydrolyze the intercellular matrix and the internal elastic membrane. The adventitia contains fibroblasts, elastin, and collagen. The *vasa vasora* and sympathetic and parasympathetic nerve fibers are contained in the adventitia. The arterial wall is a highly dynamic and responsive environment with the various cellular constituents of different layers communicating through complex signaling circuits. Arteries undergo a staggering series of changes, both biochemically and physiologically, during all stages of atherogenesis.

Endothelial Cell Function and Dysfunction

Endothelial cells line the luminal surface of blood vessels, provide barrier functions to control what enters and exits the arterial wall, and carry out a number of other specialized roles. Endothelial continuity and barrier function are established by tight junctional complexes between cells [21]. These "gap" junctions also facilitate communication between endothelial cells [22]. The endothelium controls vascular tone by producing nitric oxide. Nitric oxide (NO) is produced by endothelial nitric oxide synthase (eNOS) using arginine as a nitrate donor. Nitric oxide production is activated by bradykinin, acetylcholine, and substance P [23]. Once formed, NO diffuses down along a

concentration gradient into the media and activates soluble guanylate cyclase, an enzyme that catalyzes the production of cyclic 5′-guanylate monophosphate (cGMP) [23]. As intracellular cGMP levels increase, smooth muscle cells relax, thereby promoting vasodilatation. Endothelial cells produce other vasodilatory substances as well, including prostacyclin (prostaglandin I_2) and endothelium-derived hyperpolarizing factor [24]. It is not yet established how much each of these molecules contributes to vasodilatory input at any given time or in response to local physiologic or pathophysiologic change.

Under normal conditions, the endothelium establishes an antithrombotic surface by producing (1) tissue plasminogen activator (tPA), an enzyme that converts plasminogen to plasmin, a thrombolytic enzyme that hydrolyzes fibrin [25], and (2) thrombomodulin and heparin sulfate, both of which antagonize the activity of thrombin. Prostacyclin and NO inhibit platelet activation and aggregation along the endothelial surface [26].

When endothelial cells are exposed to increased levels of atherogenic lipoproteins, elevated systemic resistance, tobacco-derived toxins, inflammatory mediators, oxygen free radicals, increased serum concentrations of glucose, oscillatory shear stress, or turbulent blood flow, they become dysfunctional [27–29]. Endothelial cell dysfunction (ECD) is a truly systemic disorder [30] and is characterized by a number of pathophysiological changes:

1. Nitric oxide production decreases [31].
2. The endothelial surface becomes more prothrombotic because the production of tPA and prostacyclin decreases and biosynthesis of plasminogen activator inhibitor (PAI; an inhibitor of tPA and fibrinolysis) increases [32].
3. The barrier function becomes impaired as the tightness of junctional complexes is adversely impacted [33].
4. Production of the vasoconstrictor endothelin-1 increases which not only increases vascular resistance but also induces adverse remodeling of the vessel wall [34].
5. The expression of adhesion molecules increases [35–37].

Adhesion molecules promote the binding, rolling, and transmigration of inflammatory white blood cells, such as monocytes and lymphocytes, along the endothelial surface and include vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and a variety of selectins (e.g., E, P, and L) [38, 39] (Fig. 2.1). As monocytes bind to the luminal surface of endothelial cells, they can gain access into the subendothelial space by homing in on a gradient of monocyte chemoattractant protein-1 (MCP-1) [40–42]. Monocytes can cross the endothelial barrier by either (1) rearranging their cytoskeleton and changing their shape (*diapedesis*) in between adjacent endothelial cells (*paracytosis*) or (2) moving directly through an endothelial cell (*transcytosis*) [38, 43, 44]. Monocytes taken up into the vessel wall can then take up residence in the subendothelial space, transform into macrophages, and create an inflammatory nidus within the arterial wall (Fig. 2.2). Different subpopulations of macrophages (M1 or M2) can then either scavenge lipids or phagocytose apoptotic debris, generate cytokines that potentiate or inhibit inflammation, or engage in other specialized functions as needed during atheromatous lesion initiation, progression/expansion, or regression [45, 46].

In addition to promoting vasodilatation, NO is critical to the inhibition of several mechanisms fundamental to atherogenesis. Nitric oxide decreases the adhesion of platelets to endothelium [47]. In addition to promoting thrombus formation, platelets stimulate intravascular inflammation by functioning as a source of such inflammatory mediators as a platelet-derived growth factor, thrombospondin, platelet factor 4, and transforming growth factor-β, among others [48]. Nitric oxide also inhibits (1) the migration of smooth muscle cells from the media into the subendothelial space, an early event in atherogenesis, and (2) intercellular matrix synthesis and deposition [49]. The intercellular matrix material is believed to be responsible for lipoprotein trapping in the subendothelial space [50, 51]. Reduced NO production is highly correlated with atherogenesis [52].

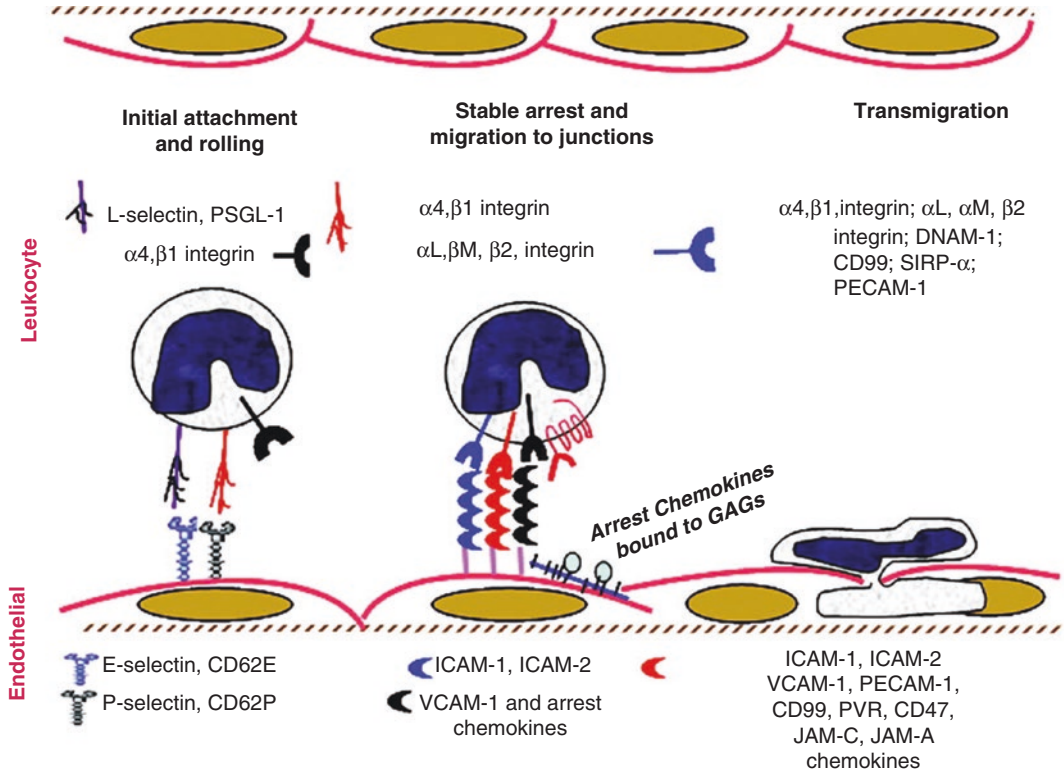

Fig. 2.1 Leukocyte recruitment from blood into the subendothelial space. Complex orchestration of cell attachment and diapedesis with sequential expression of different integrins, selectins, and adhesion molecules. Initial attachment and rolling, arrest, and migration to cell-cell borders and transmigration across the vascular endothelium of a monocyte. Monocytes attach via selectin-mediated mechanisms along with contributions from the *a*4 and ß2 integrins binding to their ligands VCAM-1 and ICAM-1, respectively. The next step is stable arrest; ß2 integrins become activated by arrest chemokines and trigger cell arrest at or near cell-cell junctions. Monocytes then migrate to junctions and transmigrate across the vascular endothelium at both junctional and non-junctional locations. The symbols used to represent adhesion molecules in endothelial cells are identified below each component of the figure. (From Rao et al. [38]. Reproduced with permission)

Angiotensin II (AII) is an important mediator of hypertension and is produced from angiotensin I (AI) via proteolytic hydrolysis by angiotensin-converting enzyme (ACE). Dysfunctional endothelium increases its expression of the AT1 receptor, the binding site for AII. Activation of AT1 by AII increases the activity of such enzymes like xanthine oxidase and NAD(P)H oxidase [53, 54]. These enzymes increase oxidative stress by increasing the production of reactive oxygen species (ROS), such as superoxide anion, hydroxyl ions, and hydrogen peroxide [55, 56] (Fig. 2.3). The ROS are directly toxic to the endothelium, quench NO (forming peroxynitrite anions), and can oxidize and peroxidize the lipids and phospholipids in lipoproteins, thereby rendering them more atherogenic. AII also promotes smooth muscle cell proliferation and migration as well as increased fibroblast collagen production and deposition. This addition of collagen leads to the loss of compliance/reduced elasticity of the artery. Endothelial cell dysfunction as measured by impaired vasoreactivity in response to an acetylcholine or methylcholine challenge [57, 58] and increased expression of PAI-1 are indicators of worse prognosis in patients at risk for cardiovascular events [59]. Endothelial function is improved by increased exercise [60] as well as

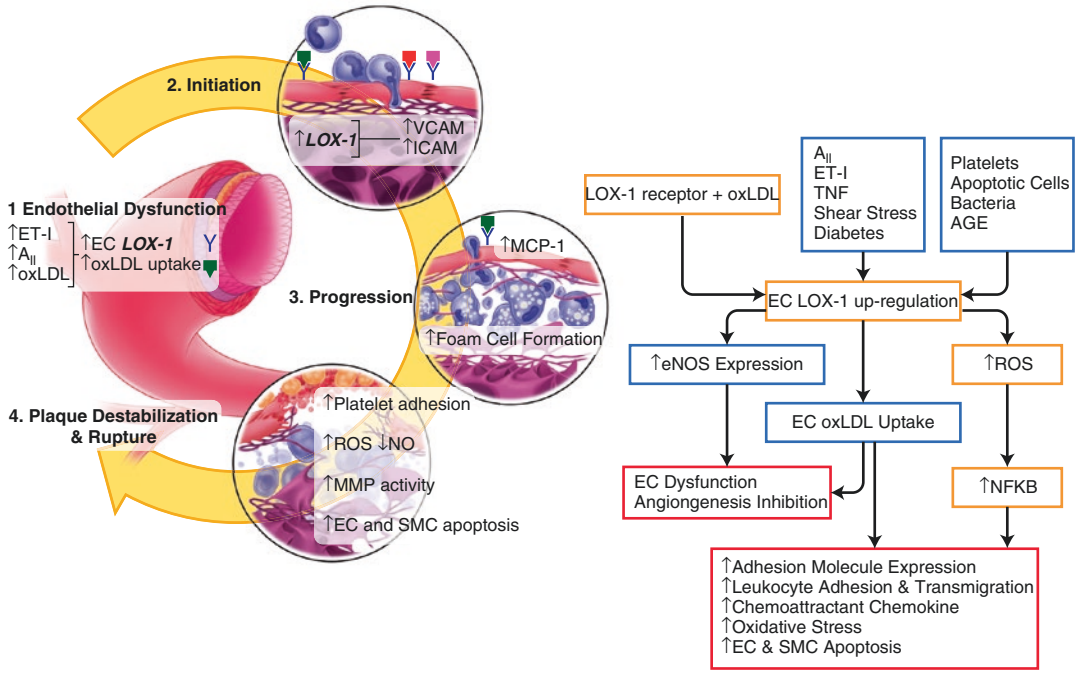

Fig. 2.2 LOX-1 and inflammation. LOX-1 plays a role in the initiation, progression, and destabilization of atherosclerotic plaques. The steps in atherogenesis it impacts are shown on the left and summarized in greater detail on the right. LOX-1 binding and signaling initiate a series of molecular and histologic events that end in vascular occlusion and ischemic injury. Abbreviations: ET-1 endothelin-1, AII angiotensin II, VCAM-1 vascular cell adhesion molecule, MCP-1 monocyte chemoattractant protein-1, MMP Matrix metalloproteinase, NO nitric oxide, oxLDL oxidized low-density lipoprotein, TNF tumor necrosis factor, NFKB nuclear factor kappa B, EC endothelial cells, SMC smooth muscle cells, ROS reactive oxygen species, eNOS endothelial nitric oxide synthase, and AGE advanced glycation end products. (From Szmitko et al. [79]. Reproduced with permission)

pharmacologic intervention with statins (3′-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor) [61] and angiotensin-converting enzyme (ACE) inhibitors [62].

Receptors of Advanced Glycosylated End Products

Patients with insulin resistance, metabolic syndrome, and diabetes mellitus have impaired glucose tolerance and hyperglycemia. Hyperglycemia correlates with increased formation of arterial advanced glycosylated end products (AGEs) [63]. The AGEs represent the nonenzymatic modification of lysine residues in enzymes, proteins, and lipoproteins with the formation of glucose adducts [64]. The formation of AGEs activates the inflammatory cascades regulated by nuclear factor Kappa-B and activator protein-1 [63, 65]. In addition, the formation of AGEs also correlates with the following:

1. Lipoprotein glycosylation (rendering LDL particles more atherogenic and compromising high-density lipoprotein particle function)
2. Endothelial dysfunction with reduced nitric oxide availability, increased adhesion molecule expression, increased procoagulant production, and heightened oxidative tone
3. Increased collagen cross-linking, leading to reduced vessel wall compliance
4. Increased subendothelial intercellular matrix deposition, increasing likelihood of atherogenic lipoprotein trapping
5. Increased leukocyte infiltration and inflammatory mediator expression, among other effects [66]

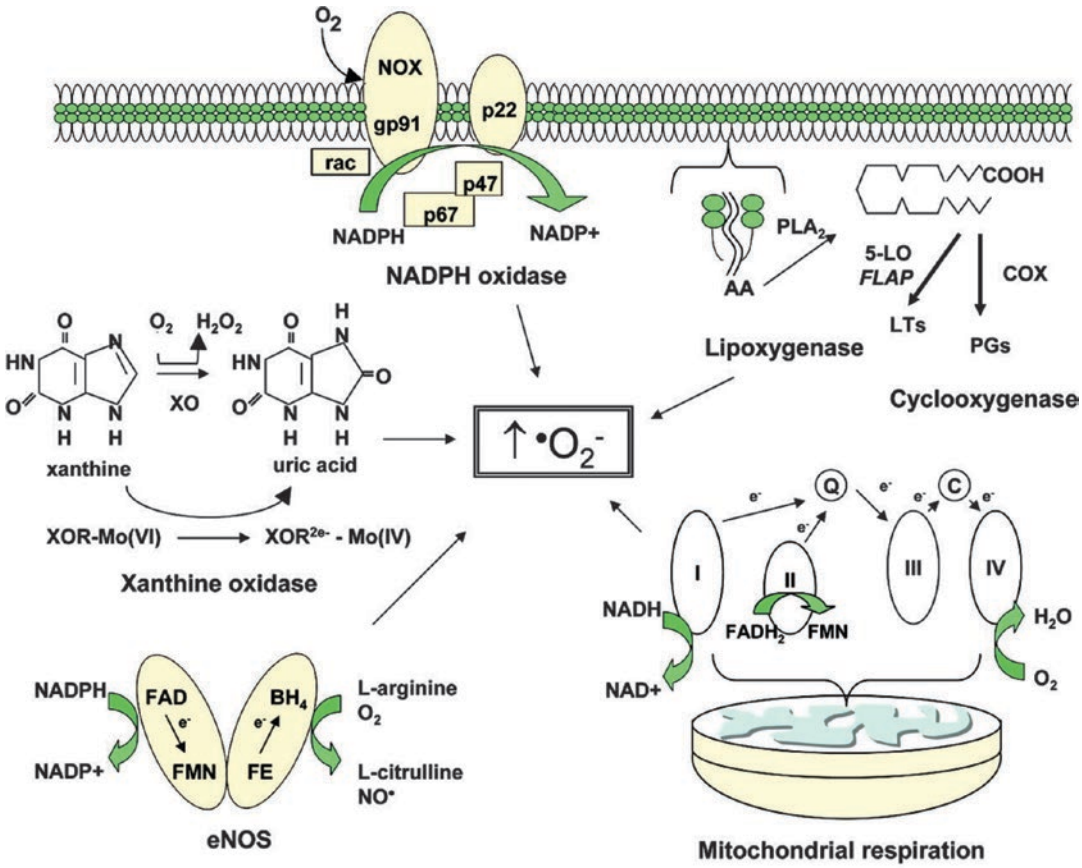

Fig. 2.3 Metabolic and enzymatic sources of superoxide anion in the vasculature. Superoxide anion (•O2⁻) is formed by several metabolic and enzymatic sources within the cell. NADPH oxidase is composed of multiple membrane-bound and cytoplasmic subunits. The enzyme is activated when the cytoplasmic subunits p67 and p47 and the small G-protein Rac assemble with the membrane-bound NOX (vascular homolog of gp91phox) and p22phox. NADPH oxidase uses NADPH as a substrate and, in vascular cells, is considered an important source of reactive oxygen species (ROS) generation. The lipoxygenases and cyclooxygenases (COX) generate ROS indirectly by promoting the formation of inflammatory mediators. Arachidonic acid (AA) that is cleaved from the cell membrane by phospholipase A2 (PLA2) is then metabolized by 5-lipoxygenase (5-LO) in the presence of its accessory protein (FLAP) to form leukotrienes (LTs). AA is also metabolized by the cyclooxygenases to form members of another family of inflammatory mediators, the prostaglandins (PGs). Mitochondria also generate superoxide as electrons are transferred from complex I to cytochrome oxidase during normal cellular respiration. Xanthine oxidase (XO), which converts hypoxanthine and xanthine to uric acid, is an additional source of ROS. As xanthine is converted to uric acid, two electrons are donated to molybdenum (Mo) at the active site of the enzyme, thereby reducing it from Mo(VI) to Mo(IV). Finally, endothelial nitric oxide synthase (eNOS), when substrates or cofactors are not replete, uncouples to generate superoxide in preference to NO. Abbreviations: Q coenzyme Q, C cytochrome C, NAD nicotinamide adenine dinucleotide, FAD flavin adenine dinucleotide, FMN flavin mononucleotide, FE heme iron, BH4 tetrahydrobiopterin. (From Leopold and Loscalzo [56]. Reproduced with permission)

The hyperglycemic milieu is particularly injurious and stimulates a broad swath of proatherogenic influences. In the setting of insulin resistance, there is increased visceral organ steatosis, especially in the liver, pancreas, and epicardium [67]. Epicardial fat pad volume expansion in the setting of insulin resistance loads the epicardium with dysfunctional fat surrounding coronary arteries. This dysfunctional fat is a source of interleukins, cytokines, and growth factors that shower the coronary tree and increase risk for atherosclerotic disease [68].

The Role of Monocytes and Lymphocytes

Monocytes that have become resident in the subendothelial space can undergo several histologic transitions. When exposed to macrophage colony-stimulating factor (M-CSF), the monocyte converts into a macrophage. Macrophages are one of the earliest histologic substrates of atherogenesis. Macrophage egress from the vessel wall can be inhibited by the neural guidance factor netrin-1 and VCAM-1; egress can be potentiated by lymphatic channels and high-density lipoprotein particles [69]. The fatty acids and phospholipids of low-density lipoprotein particles can undergo oxidation via such enzymes as myeloperoxidase [70], NADPH oxidase, and a variety of lipoxygenases [71] (Fig. 2.4). These oxidized phospholipid species are complex and potentiate inflammation and oxidation (Fig. 2.5). In addition, in the setting of hyperglycemia, lipoproteins can undergo glycation [72].

Exposure to oxidatively modified or glycated low-density lipoprotein particles trapped by intercellular matrix proteins in the subendothelial space [73] stimulates macrophages to upregulate the expression of a number of scavenger receptors on their surface [74]. There are a large number of these scavenger receptors and include multiple types of scavenger receptor A (types I-III) [75], CD36 [76], lectin-like oxidized LDL receptor-1 (LOX-1) [77], and scavenger receptor for phosphatidylserine and oxidized LDL (SR-PSOX) [78], among others. LOX-1 is also expressed by endothelial cells and smooth muscle cells. In the setting of increased oxidized LDL (oxLDL) exposure, endothelial cells upregulate LOX-1; the uptake of oxLDL is toxic and potentiates endothelial dysfunction and adhesion molecule expression [79].

The oxidation of phospholipids in LDL particles generates the formation of oxidation specific epitopes recognized by scavenger receptors [80, 81]. These include oxidized sn-2 fatty acids that terminate in γ-hydroxy-α, β-unsaturated carbonyl groups or 1-palmitoyl-2-(5′-oxovaleroyl)-sn-glycero-3-phosphocholine (Fig. 2.5). Scavenger receptors promote the binding and uptake of atherogenic lipoproteins into the intracellular space of the macrophage. As more and more lipids are taken up, the macrophage develops lipid inclusion bodies and becomes a "foam cell." [82] Foam cells produce a variety of cytokines, matrix metalloproteinases, ROS, and tissue factors [83]. Smooth muscle cells can undergo transformation into macrophages and, as they scavenge lipid and lipoprotein, can also form foam cells [84]. Smooth muscle cell transformation occurs secondary to reduced expression of myocardin and the microRNAs miR143/145 [85]. Oxidized phospholipids can also stimulate the hyperphosphorylation of VE-cadherin, a critical protein for maintaining endothelial gap junctions [86]. Gap junction function deteriorates as the VE-cadherin dissociates from the proteins β-catenin and paxillin [87].

Tissue factor is a procoagulant that promotes platelet aggregation on the surface of ruptured atheromatous plaques [88]. The MMPs can destabilize atheromatous plaque by hydrolyzing the matrix proteins which reinforces its structural integrity (Fig. 2.6). As MMPs degrade extracellular matrix material, such degradation products as integrin-binding fibronectin, hyaluronan, and heparan sulfate can trigger immune and proinflammatory responses [81, 89, 90]. Smooth muscle cells also produce MMPs as they break down the internal elastic lamina in order to access the intima [20]. Ultimately, foam cells can coalesce to form fatty streaks. As fatty streaks increase in volume and more cellular debris accumulates, a frank atheromatous plaque evolves.

Foam cells possess measures of self-defense. Macrophages are capable of effluxing excess intracellular lipids into the extracellular space. Intracellular cholesterol can be mobilized and exported onto HDL particles via scavenger receptor B-I (SR-BI), or two ATP-binding membrane cassette transport proteins termed ABCA1 and ABCG1 [91]. In addition, the macrophage can produce and secrete apoprotein E (apoE) which, when externalized, can bind to ABCA1 and drive cholesterol externalization, apoE lipidation, and lipoprotein biogenesis [92, 93]. If these defenses are overwhelmed by excess lipid trapped in the subendothelial space, then foam cell develop-

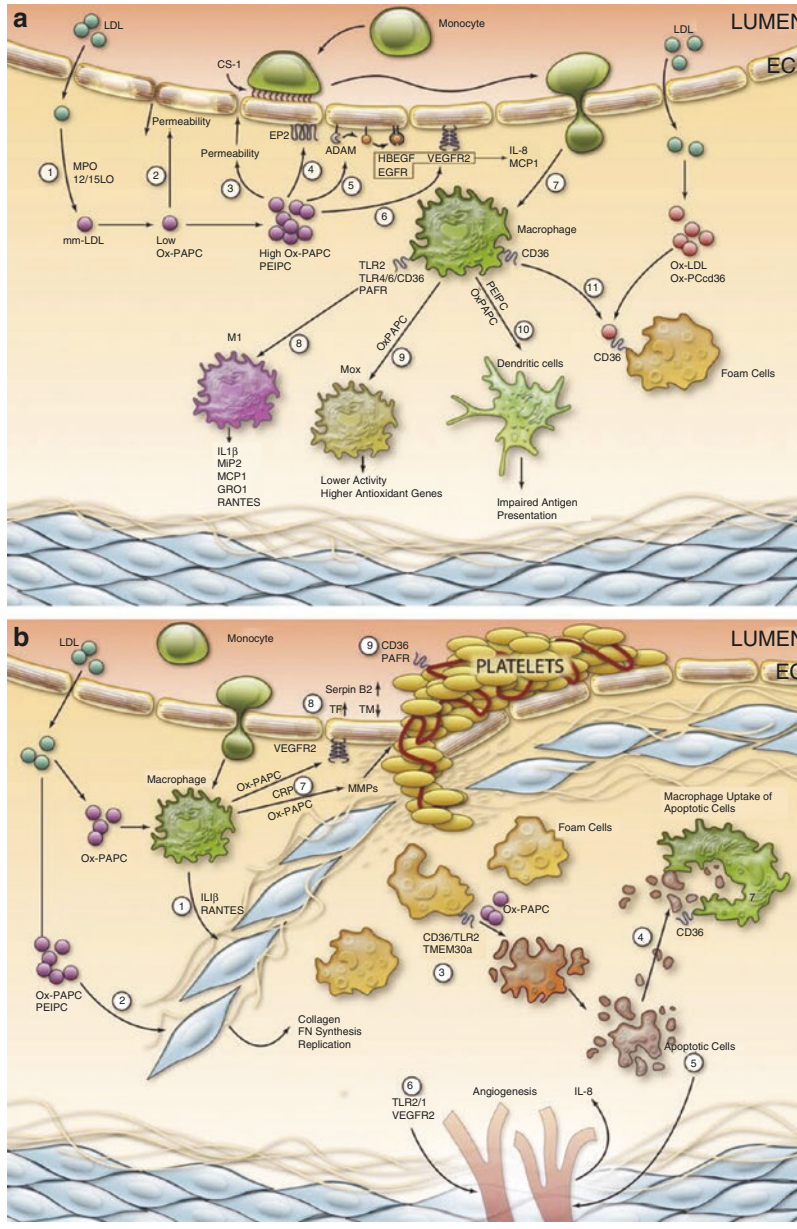

Fig. 2.4 Model of oxidized phosphatidylcholine-containing phospholipids (Ox-PL) regulation of atherosclerosis. (**a**) Early lesions: (1) LDL enters the vessel wall and is oxidized by myeloperoxidase (MPO) and 12/15 lipoxygenase (LO) to form modified LDL which contains oxidation products including oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphocholine (Ox-PAPC). (2) Low doses of Ox-PAPC decrease the permeability of the endothelial cell (EC) monolayer by forming adherens junctions. (3) Higher levels of Ox-PAPC cause a strong increase in monolayer permeability because of junction breakdown and stress fiber formation, resulting in increased entry of LDL into the vessel wall. (4) Ox-PAPC binds to the E-type prostaglandin receptor (EP2) receptor, causing the deposition of connecting segment 1 (CS-1) fibronectin on the apical surface which binds monocytes. (5) Ox-PAPC activates specific a disintegrin and metalloproteinases (ADAMs) to cause the release of active heparin-binding epidermal growth factor (HBEGF) and activation of epidermal growth factor receptor (EGFR), leading to interleukin (IL)-8 and monocyte chemotactic protein (MCP)-1 synthesis. (6) Ox-PAPC also activates vascular endothelial growth factor receptor 2 (VEGFR2), leading to IL-8 and MCP-1 synthesis. (7) These chemokines facilitate the entry of monocytes into the vessel wall. (8) Oxidized phosphatidylcholine-containing phospholip-

(continued)

ids (Ox-PL) acting on Toll-like receptor (TLR)2, TLR4/6/cluster determinant 36 (CD36), and platelet-activating factor (PAF) receptor cause some monocytes to differentiate into M1 macrophages producing chemokines. (9) Ox-PAPC causes differentiation of some macrophages into Mox, which have high levels of antioxidant enzymes and lower chemokine syntheses. (10) Ox-PAPC causes the differentiation of some monocytes into dendritic cells with an impaired presentation of lipid antigens. (11) Macrophages further oxidize LDL to form Ox-LDL. Ox-PCCD36 acting on CD36 in the presence of Ox-LDL causes foam cell formation. (**b**) Advanced lesions: (1) In the presence of Ox-PAPC and PAF-like lipids, macrophages make IL-1 beta (IL-1β) and regulated upon activation, normal T-cell expressed, and secreted (RANTES). (2) These chemokines and a direct effect of Ox-PAPC on smooth muscle celsl (SMC) cause the migration and proliferation and matrix production of SMC. These SMC cover the foam cells that accumulate under the endothelium. (3) The interaction of Ox-PAPC with CD36/TLR2 and with unfolded protein response (UPR) activators and the interaction of PAF-like lipids with transmembrane protein 30A (TMEM30a) cause macrophage apoptosis. (4) Oxidized phosphatidylserine-containing phospholipids (Ox-PS)/ PCCD36 in the apoptotic cell membrane bind to CD36 in macrophages, leading to macrophage uptake of the apoptotic cells. (5) Some apoptotic fragments stimulate EC to make IL-8, an angiogenic cytokine. (6) CEP activation of TLR2/TLR1 causing integrin activation and Ox-PAPC acting to increase VEGFA cause angiogenesis of adventitial vessels into the media and intima. (7) C-reactive protein (CRP) and Ox-PAPC interacting with CD36 stimulate macrophage production of metalloproteinase. This weakens the plaque and can lead to plaque rupture. (8) Ox-PAPC activation of VEGFR2 increases tissue factor synthesis in the endothelium. Ox-PAPC also causes increases in Serpin B2 and a decrease in thrombomodulin. (9) Ox-PCCD36 acting on CD36 and PAF-like lipids acting on PAFR cause increased aggregability of platelets. (From Lee et al. [86]. Reproduced with permission)

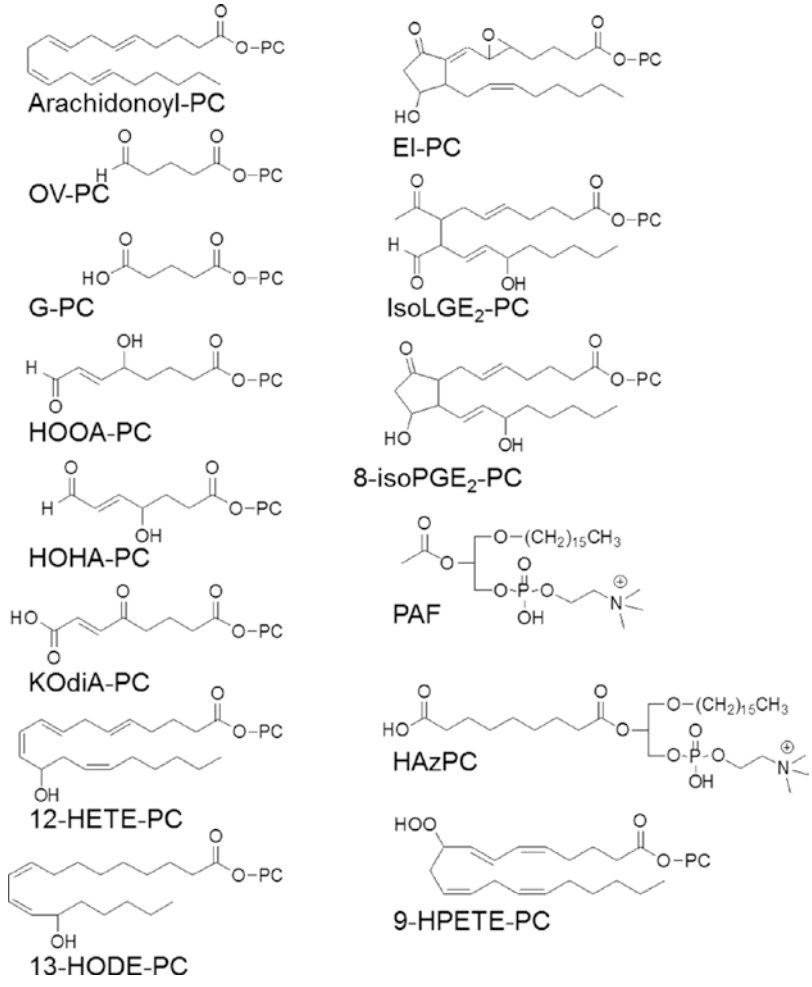

Fig. 2.5 Oxidized phosphatidylcholine-containing phospholipids (OX-PL) lipids**.** PC, 1-acyl-2-lyso-sn-glycero-3phosphatidylcholine. Only the sn-2 position composition is shown for all Ox-PL except those forming an ether bond at the sn-1 position. Abbreviations: PAF platelet-activating factor, HAz-PC hexadecylazelaoyl PC, 13-HODE-PC 1-palmitoyl-2-(13(S)-hydroxy-(9Z,11E) octadeca-9,11-dienoyl)-sn-glycero-3-phosphocholine. (From Lee et al. [86]. Reproduced with permission)

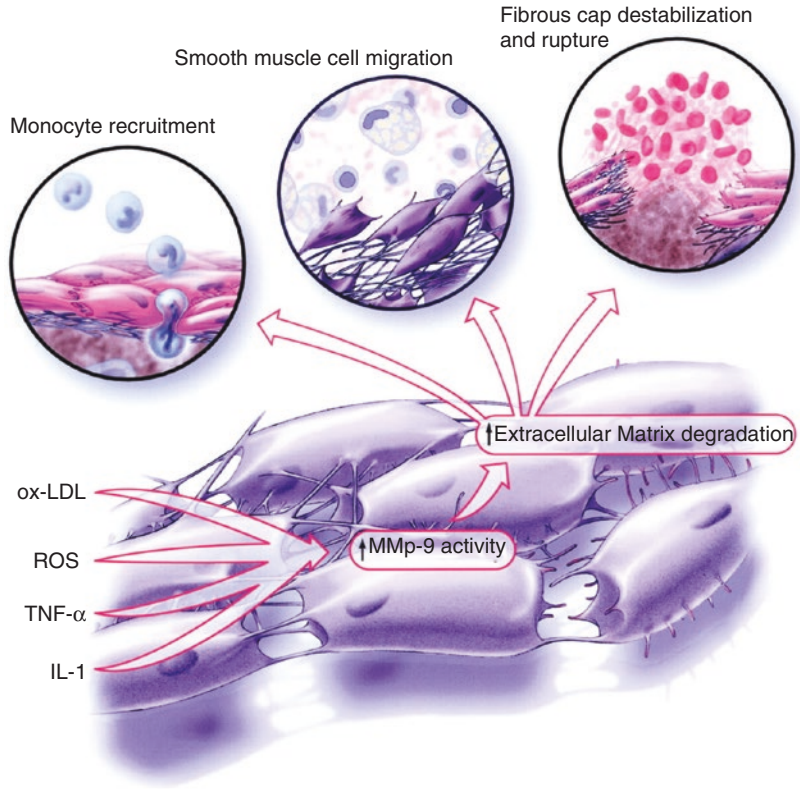

Fig. 2.6 MMP-9, from plaque progression to destabilization. MMP-9 degrades the basement membrane of the arterial wall to facilitate monocyte migration into the plaque and SMC migration to form the fibrous cap. Excessive MMP-9 activity eventually leads to the degradation of the fibrous cap, plaque instability, and plaque rupture. Abbreviations: IL-1 interleukin-1, ROS reactive oxygen species, TNF-α tumor necrosis factor alpha, MMP-9 matrix metalloproteinase-9, oxLDL oxidized low-density lipoprotein. (From Szmitko et al. [79])

ment progresses with increasing lipid inclusion body volume and resultant toxicity [94].

T lymphocytes and mast cells also participate in vascular inflammation and atherogenesis. T cells follow a gradient of chemoattractants (inducible protein-10, interferon-inducible T-cell α-chemoattractant, and monokine induced by interferon-γ) into the subendothelial space [41, 95]. These chemoattractants can bind to CXCR3, a chemokine receptor on the surface of T cells. When a T cell binds oxidatively modified LDL to an antigen receptor it can undergo differentiation into T helper cells, such as TH1 and TH2. TH1 cells potentiate inflammation by producing interleukin-1, interferon-γ, and tumor necrosis factor. TH2 cells produce anti-inflammatory cytokines, such as interleukins −4 and −10. TH1 cells predominate in atheromatous plaques and stimulate inflammation. Following antigen binding and presentation, T cells stimulate macrophage production of MMPs and cytokines.

Lymphocytes also infiltrate and become organized in the vascular adventitia [96, 97]. Adventitial aortic tertiary lymphoid organs (ATLOs) and T cell aggregates associate with more severe atherosclerotic plaques. An ATLO is composed of a nodular center composed of B lymphocytes and dendritic cells surrounded by T lymphocytes. B lymphocytes can be activated to produce antibodies after antigen presentation by dendritic cells, thereby mounting an immune response. There is significant communication between the endothelium and adventitia, and it is believed that ATLOs and organized T cell aggregates play a significant role in atherogenesis [98]. The vasa vasora and small medial conduits mediate the transfer of immune cells, cytokines, and interleukins between the intima and adventitia (Fig. 2.7).

Activated mast cells contribute to atherogenesis and enter the subendothelial space in response to eotaxin exposure [99]. Mast cells secrete two serine peptidases, tryptase and chymase [100]. Chymase catalyzes the intravascular conversion

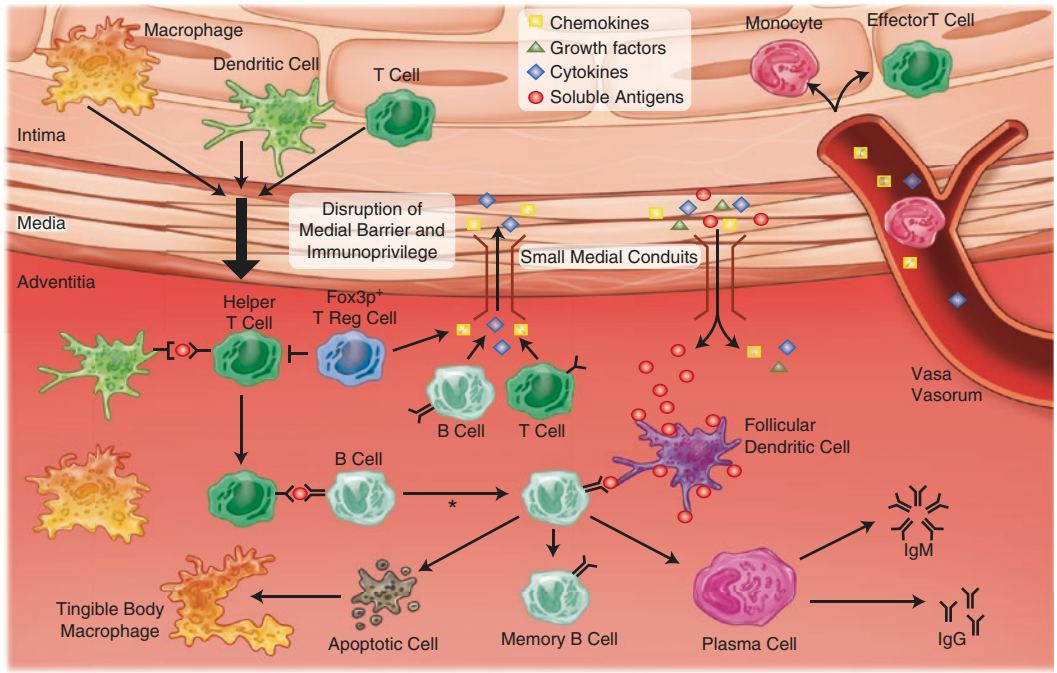

Fig. 2.7 Arterial adventitia and its role in atherogenesis. Lymphocytes, macrophages, dendritic, cells, and plasma B cells can be organized in the adventitia of arteries. Small medial conduits facilitate the passage of cytokines, chemokines, soluble antigens, and growth factors from the adventitia into the media. The vasa vasora can facilitate communication between cells of the adventitia and those of the intima, including endothelium. These communication patterns can promote atherogenesis by stimulating inflammatory and phagocytic cell recruitment, smooth muscle cell migration, and the mounting of an innate immune response. (From Campbell et al. [98]. Reproduced with permission)

of AI to AII, and tryptase activates MMPs. Both enzymes thus not only contribute to early events in atherogenesis but also can induce instability in established plaque. Mast cells also secrete histamine, which promotes increased vascular permeability.

Role of Neutrophils

Neutrophils have evolved a broad-based capacity for biochemically combating infectious organisms by secreting proteases, ROS, and antimicrobial proteins. However, recent investigation also supports multiple roles for neutrophils in atherogenesis. Within the subendothelial space, neutrophils can produce an array of collagenases, elastases, and other matrix metalloproteinases that can hydrolyze and degrade the intercellular matrix material of plaque and its fibrous cap, thereby weakening them and rendering them more prone to rupture [101]. Neutrophils also elaborate myeloperoxidase and ROS in the subendothelial space which are cytotoxic and oxidize trapped lipoproteins [102]. Neutrophils entering the subendothelial space also potentiate injury by releasing (1) four different subsets of granules containing preformed proteases, pro-oxidative enzymes, and cytokines whose release is precisely timed in response to conditions in the prevailing histologic milieu and (2) and leukotrienes such as LTB4, a potent chemoattractant [103].

A more recently elucidated pathway by which neutrophils can promote atherogenesis is by forming neutrophil extracellular traps (NETs) [104] (Fig. 2.8). NETs are produced by suicidal neutrophils and represent an extruded reticular structure composed of decondensed chromatin as

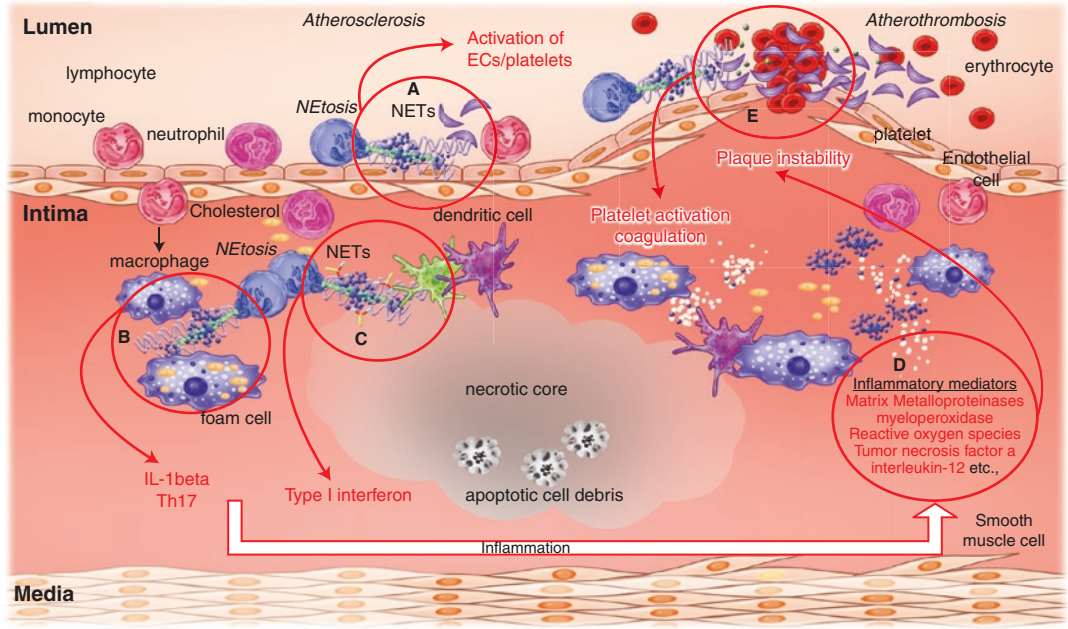

Fig. 2.8 Neutrophil extracellular traps (nets) in atherosclerosis and atherothrombosis. (**a**) Neutrophils netting in the arterial lumen along the endothelial surface activates endothelial cells, platelets, and other leukocytes, inducing an inflammatory nidus and endothelial dysfunction. (**b**, **c**) NETs may stimulate T helper cells to secrete IL-1β and potentiate a type I interferon response, which boosts leukocyte activation and the intensity of inflammation. (**d**, **e**) The proinflammatory milieu promotes plaque instability and rupture. In the setting of acute plaque rupture, NETs can participate in thrombus formation by activating the coagulation cascade with overlying thrombus formation and arterial occlusion. (From Doring et al. [104]. Reproduced with permission)

well as nuclear, granular, and cytosolic proteins. NETs represent a type of mechanism by which endothelial cells can be exposed to sudden, very high concentrations of proinflammatory mediators. NETosis or the process of NET formation can be induced by ROS, cytokines, cholesterol crystals, and activated platelets [105–108]. In addition to nucleic acids, the molecular constitution of NETs contains a complex proteome which includes histones, proteases, lysosomal cathepsins, α-defensins, and myeloperoxidase, among other proteins and enzymes [104]. NETS are prothrombotic and cytotoxic.

Role of Platelets

Platelets are nonnucleated cells arising from parent megakaryocytes and mediate clot formation in concert with coagulation pathways. Platelets potentiate atherogenesis in multiple ways. Platelet α-granules contain a host of cytokines, chemokines, growth factors, and enzymes that can be mobilized and secreted in response to extracellular stimuli [109]. Platelets interact with endothelial cells and leukocytes according to the following mechanisms:

1. Platelets adhere to dysfunctional endothelial cells by binding to either (a) ICAM-1 via the glycoprotein 2b/3a receptor and fibrinogen or (b) selectin P via glycoprotein 1b [109, 110].
2. Thrombus formation along the endothelial surface is modulated in a bidirectional manner. Endothelial cells secrete nitric oxide and prostacyclin, which inhibits platelet activation and aggregation. Endothelial cells can also attenuate ADP availability by releasing CD39 ectonucleotidase, which hydrolyzes ADP to AMP and phosphate. Platelets can secrete nitric oxide which inhibits endothelial P-selectin expression, reduces platelet

recruitment for clot propagation, and promotes platelet dissociation [111].
3. Despite being nonnucleate, platelets effectively store messenger RNAs (mRNAs) for subsequent protein translation. In the setting of heightened inflammation, platelets can boost the inflammatory response by releasing IL-1β, among other inflammatory mediators [112, 113].
4. Inflammation can induce the coactivation of platelets and neutrophils, which leads to increased production of human neutrophil peptide-1 (HNP-1) and regulated activation of normal T cell expressed and secreted (RANTES). RANTES and HNP-1 facilitate monocyte adhesion to endothelial cells and recruitment into the arterial wall [114].
5. In addition to signal transmission by cell surface receptors and granule release, platelets can interact with endothelial cells and leukocytes by direct bilateral mRNA transmission, thereby boosting local molecular biosynthetic capacity and an inflammatory response [115].
6. Platelet microparticles also upregulate the inflammatory response. These microparticles secrete microRNAs (miRNA), which are noncoding RNAs that regulate posttranscriptional gene expression. For example, platelet-derived miRNA-320b decreases surface expression of endothelial ICAM-1 and miRNA-223 stimulates increased phagocytic activity by macrophages resident in the subendothelial space [116–118].

Clearly, the interactions of platelets with endothelial cells and other histologic components of the arterial wall and atherosclerotic plaque are complex and highly orchestrated. Much remains to be learned about these processes and how they might be therapeutically modulated.

Role of MicroRNAs

As noted above, miRNAs are noncoding RNAs that regulate posttranscriptional gene expression. MicroRNAs are highly conserved and bind to the 3′ untranslated region of messenger RNA (mRNA) transcripts, resulting in "RNA silencing." [119] They are produced and secreted by a large variety of cells. MicroRNAs secreted into the circulation are resistant to the activity of plasma RNases, and they can impact the expression of molecules in target cell types. MicroRNAs can be transported in the plasma on microparticles, HDL particles, or bound to the protein Argonaute2 [120]. Distinct patterns of circulating miRNAs have been characterized in the setting of myocardial infarction, heart failure, and diabetes mellitus [120–123]. Specific molecular signatures of miRNAs are also apparent in the setting of CAD [124]. The miRNAs do not unexpectedly have a very complex relationship with atherogenesis, with numerous miRNAs that can either stimulate or inhibit expansion of the vasa vasora, macrophage cholesterol efflux, vascular remodeling, smooth muscle cell proliferation and migration, endothelial cell activation, and monocyte and T-cell differentiation and activation, among other functions [125] (Fig. 2.9). Much is yet to be learned about the role of miRNAs in atherogenesis and how the modulation of these regulators of gene expression might be put to therapeutic use.

Role of Increased Oxidative Tone

Myeloperoxidase, lipoprotein-associated phospholipase A2, xanthine oxidase, NADPH oxidase, cyclooxygenase, and 5′-lipoxygenase are all found in atheromatous plaque and promote ROS production and oxidative lipoprotein modification [56, 126, 127]. The ROS include superoxide anion [128], hydroxyl radicals, peroxynitrite radicals, and hydrogen peroxide [129] (Fig. 2.3). Enzymes such as glutathione peroxidase, the thioredoxins, paraoxonase, and superoxide dismutase are responsible for metabolizing ROS to less reactive species. Deficiencies in anti-oxidative enzymes can be associated with increased atherogenesis. All the major cardiovascular risk factors (dyslipidemia, cigarette smoking, hypertension, diabetes mellitus) increase oxidative tone by upregulating the speciation of ROS [128]. The ROS not only can be directly cytotoxic but also

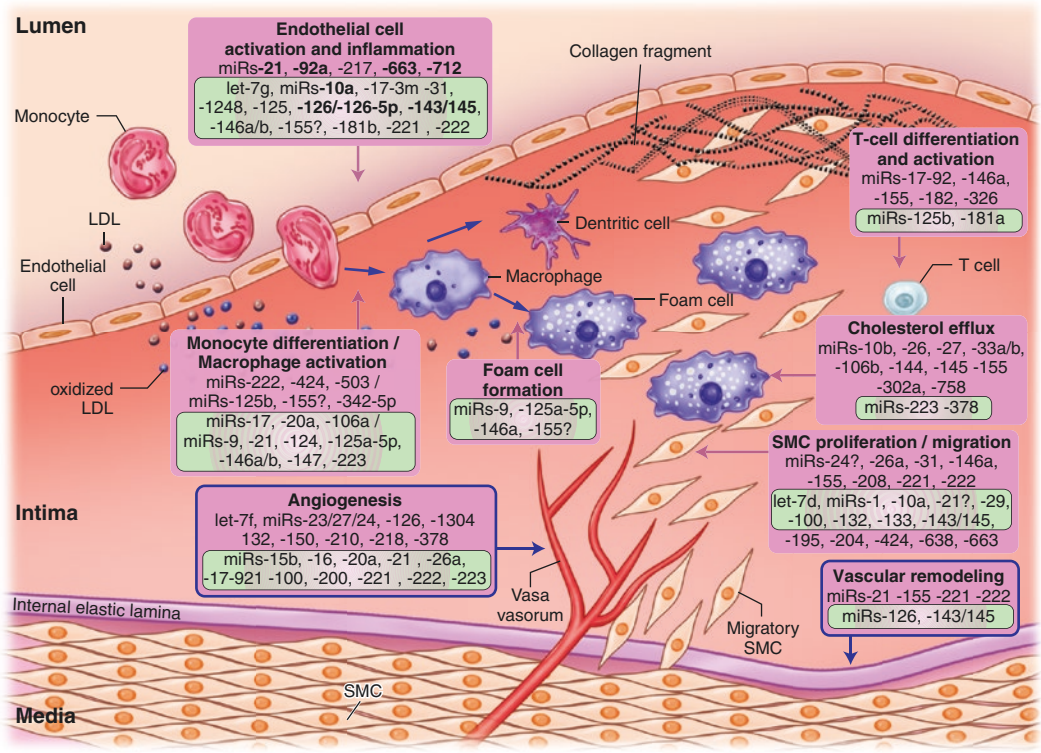

Fig. 2.9 MicroRNAs implicated in atherosclerosis. Positive/atheroprotective (in green frame) or negative/atherogenic (in red frame) effects of miRNAs on atherogenesis. Question marks indicate controversial or contradictory evidence for specific miRNAs. miRNAs in bold are regulated by blood flow/shear stress. Abbreviations: LDL low-density lipoprotein, SMC smooth muscle cell. (From Andreou et al. [125]. With permission from Elsevier)

are responsible for oxidizing and peroxidizing lipid and phospholipid within LDL particles. Lipid peroxidation products (e.g., malondialdehyde, 4-hydroxynonenal, phosphocholine of oxidized phospholipids, γ-ketoaldehydes, and 2-(ώ-carboxyethyl) pyrrole) are highly reactive [130, 131]. For example, proteins can be rendered immunogenic when they form adducts with γ-ketoaldehydes, resulting in the activation of T cells and dendritic cells [132].

Atheromatous Plaque

During the initial phases of atherogenesis, macrophage foam cells that undergo programmed cell death and turn into apoptotic bodies are efficiently cleared by macrophage dependent phagocytosis. This orderly clearance process does not promote inflammation. However, as the rate of foam cell formation and accumulation increases, the milieu within the vessel wall changes [133]. More cellular apoptosis and oncosis (ischemic death) ensues [134]. Phagocytic capacity is eventually exceeded, and the balance between foam cell apoptosis and clearance is lost, leading to progressive accumulation of lipid and apoptotic debris (Fig. 2.10). Fatty streaks progressively enlarge forming an atheromatous plaque which organizes with a lipid core and fibrous cap. More advanced lesions can have a necrotic core and can undergo calcification via the activity of a variety of osteogenic factors, including bone morphogenetic protein, osteonectin, and osteocalcin, among others [135]. Plaque that is not yet fibrosed or calcified retains some degree of plas-

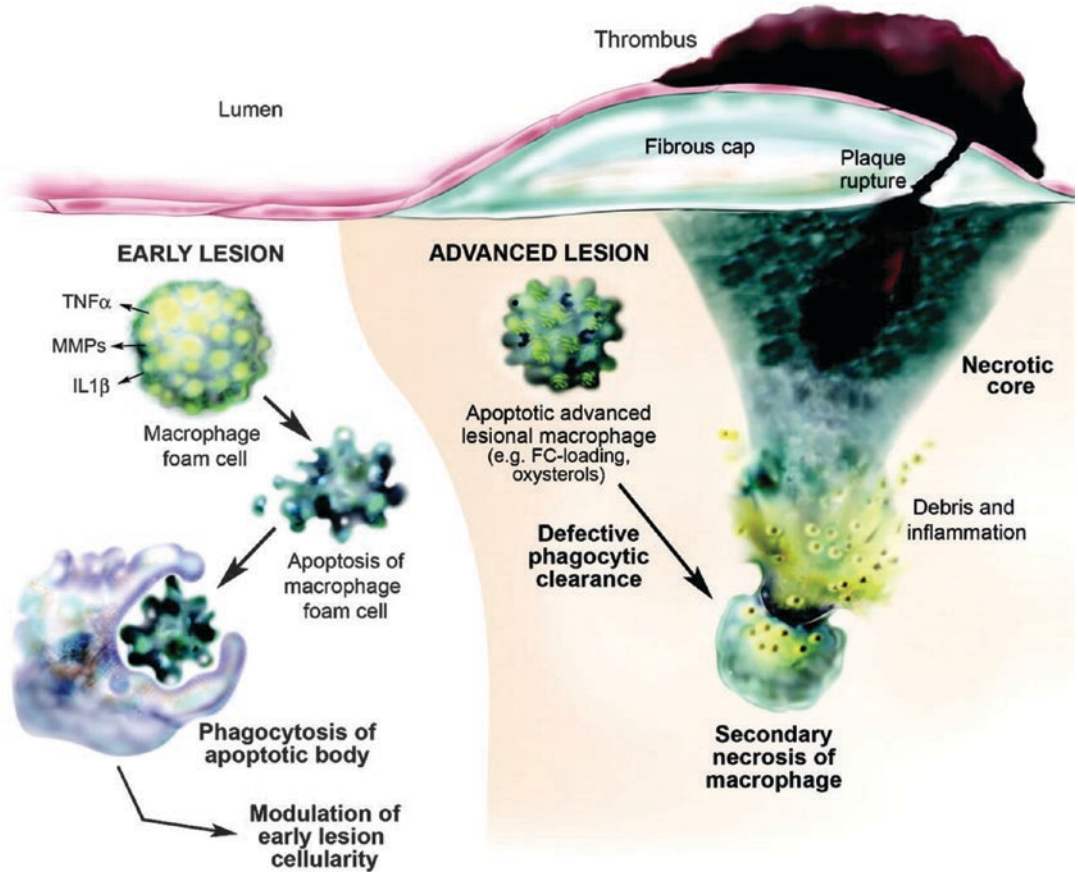

Fig. 2.10 The so-called "volcano" model of atherosclerotic plaque formation. In early atherosclerotic lesions (left), macrophage foam cells undergo apoptosis and are efficiently phagocytosed and cleared by other macrophages. This process controls lesion cellularity and rate of disease progression. However, in later lesions (right), apoptotic macrophages are not engulfed and cleared as efficiently resulting in a net accumulation of apoptotic and necrotic macrophages with the generation of a necrotic core. This leads to the mounting of an inflammatory response which can lead to plaque instability and eventual rupture. (From Tabas [133]. Reproduced with permission)

ticity, as evidenced by the observation that multiple therapeutic interventions can induce plaque regression in target lesions [136–138].

The phagocytosis of apoptotic cells and apoptotic bodies is tightly orchestrated. Apoptotic cells express a variety of "find me" (e.g., lysophosphatidylcholine, sphingosine-1-phosphate, the fractalkine CX3CL1, and adenosine 5′-triphosphate and uridine-5′-triphosphate) and "eat me" (e.g., phosphatidylserine, altered ICAM-1 epitopes on the cell surface, increased calreticulin exposure) molecules that promote phagocytic cell attraction and migration, target cell discovery, and engulfment/clearance [139, 140]. Apoptotic neutrophils express neutrophil-borne pentraxin-3 which promotes their recognition and removal by macrophages [141]. Lactadherin functions as a coupling molecule that facilitates the binding of apoptotic cell phosphatidylserine to vitronectin on phagocytic macrophages [142]. It is possible that deficiencies in these molecules may lead to impaired apoptotic cell clearance. An example of this is a deficiency in the receptor tyrosine-protein kinase MER which is associated with rapid progression and enlargement of the necrotic core in experimentally induced plaques [143].

As an atheromatous plaque evolves, the arterial wall reorganizes in a way that maintains luminal diameter and blood flow [144], a process known as positive or "Glagovian" remodel-

ing [145]. Plaque initially develops in an outward direction, producing vessel wall ectasia. It is only in the later stages of atheromatous plaque evolution that there develop progressive luminal obstruction and, ultimately, physiologically significant reductions in blood flow and oxygen delivery. Within the plaque, cellular necrosis promotes increased inflammation which accelerates atherogenesis and destabilizes plaques [146, 147]. As an illustration of just how important inflammation is in atherosclerosis, suppressing inflammation in humans with a monoclonal antibody directed against IL-1β results in a reduction of acute cardiovascular events independent of any change in serum lipoprotein levels [148, 149].

Maintaining the architectural stability of a plaque is essential to preventing acute cardiovascular events. Unstable plaques typically have large lipid cores, high inflammatory tone (characterized by increased macrophage density and increased inflammatory mediator expression), and reduced smooth muscle cell density [150]. In contrast, stable plaques are characterized by increased smooth muscle cell density, low inflammatory tone, small macrophage infiltrates, and a small lipid core. Calcification of plaque also tends to render it more stable [151, 152].

Superficial surface erosions, plaque ulceration, and frank plaque rupture expose the lipid core to blood [147, 153–156]. This exposed lipids as well as tissue factors and collagen promote platelet degranulation and aggregation, resulting in the propagation of an overlying thrombus [157]. If the thrombus completely occludes the arterial lumen, the patient experiences acute tissue ischemia. A thin fibrous cap provides less structural and tensile strength opposing plaque fracture and opening in response to a sudden stressor, such as vasospasm or hemorrhaging into the base of a plaque from injured or leaky *vasa vasora*. Hemorrhaging into the base of a plaque is an important cause of atheromatous plaque rupture. A sudden rise in the volume of a plaque can lead to the loss of architectural integrity. In addition, repetitive low volume hemorrhages into the base of a plaque secondary to leaky *vasa vasora* can lead to cumulative trauma, increased entry of leukocytes, and increased deposition of cholesterol and other lipids in the core of the plaque [158]. As erythrocytes are cleared from the plaque's interior, cholesterol from cell membranes is left behind and functions as a substrate for expansion of the plaque's lipid core (Fig. 2.11). Over time, this too can lead to plaque destabilization. The plaques that are least likely to rupture are the ones that are calcified and fibrotic.

In the statin era, it is apparent that the percentage of ACS secondary to plaque erosion rather than acute plaque rupture has been increasing [159]. Eroded plaques are described as having been denuded of endothelium and have increased neutrophils (and myeloperoxidase activity), decreased macrophage and T-cell constituents, small lipid cores, and large numbers of smooth muscle cells with dense proteoglycan and glycosaminoglycan intercellular matrix material [160–162].

A variety of coronary imaging studies suggest that culprit lesions giving rise to ACS have (1) large plaque volume, (2) large necrotic core, and (3) positive remodeling compared to plaques that remain stable [163]. Among patients suffering sudden death, more than 70% of ruptured plaques were characterized as having >75% luminal narrowing. In contrast, 5% of these cases were due to culprit lesions with <50% luminal narrowing [164]. Among patients with ST-segment elevating MI, the average luminal obstruction is 66% [165]. Typically, there is significant, rapid progression of plaque volume prior to its rupture, which can also be quite unpredictable [166]. Identifying vulnerable plaque that will eventually rupture or fissure remains a significant unsolved issue in contemporary cardiology [167].

A more recently elucidated mechanism by which plaque can rupture is from the formation of cholesterol crystals within the plaque. Recent investigation shows that cholesterol can crystallize within lesions as well as perforate the plaque surface, leading to core expansion, intimal injury, and plaque instability [168, 169] (Fig. 2.12). Oxidized LDL scavenged by the macrophage cell surface receptor CD36 correlates with cholesterol crystallization [170, 171]. Cholesterol crystals augment plaque inflammation by activating

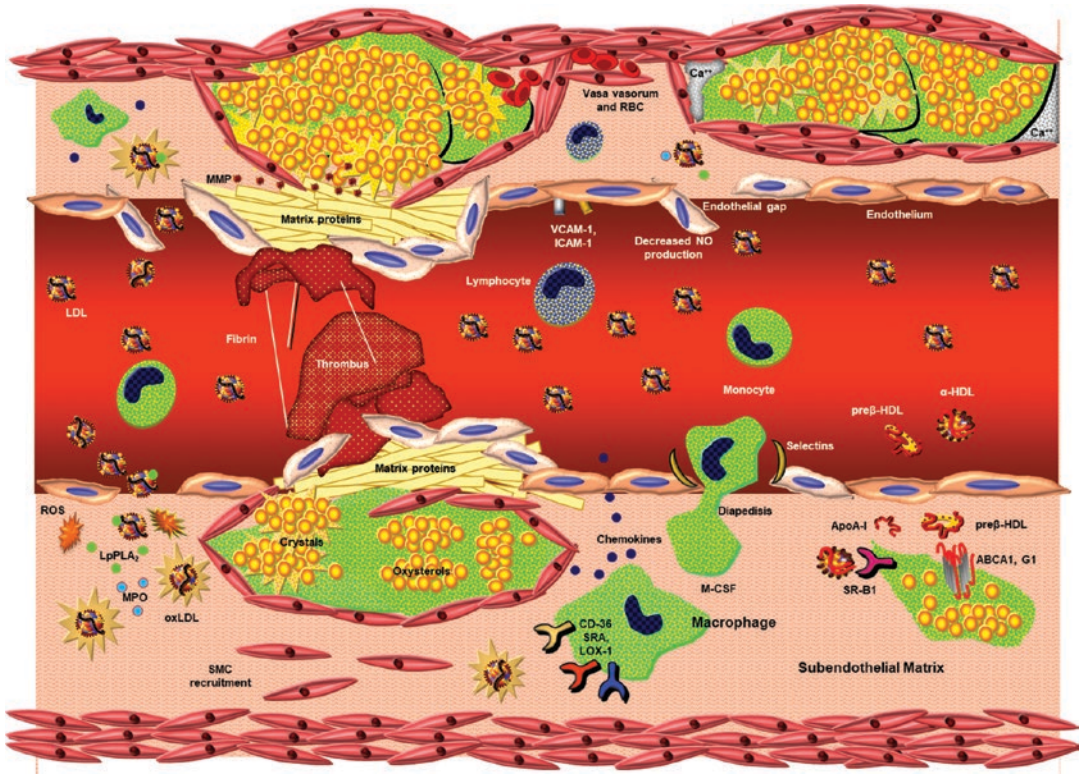

Fig. 2.11 Schematic of atherogenesis and atherosclerotic plaque rupture. *Lower right quadrant*. Monocyte in serum binds to selectins (ICAM-1, VCAM-1, selectin-P) on dysfunctional endothelium. The monocyte then reorganizes its actin cytoskeleton and traverses loosened gap junctions between endothelial cells in response to monocyte chemoattractant protein-1 (MCP). Once in the subendothelial space, it can secrete interleukins and cytokines to mount an inflammatory response. As the monocyte takes up residence, it converts to a macrophage, of which there are multiple populations. By expressing such cell surface receptors as CD36, scavenger receptor A (SRA), and lectin-like oxidized low-density lipoprotein receptor (LOX-1), macrophages scavenge oxidized low-density lipoproteins (LDL) and remnant lipoproteins. As intracellular cholesterol content increases, the macrophage becomes a progressively more lipid-enriched foam cell. In order to offload cholesterol, macrophages express the transmembrane cholesterol transport proteins ATP-binding membrane cassette transport proteins (ABC) A1 and G1, which can lipidate apoprotein A1 and spherical HDL particles, respectively. Early during atherogenesis, smooth muscle cells from the tunica media are recruited for transmigration into the intima. *Upper right quadrant*. Lymphocytes also bind to cell surface adhesion molecules and function as antigen presentation cells and a source of inflammatory mediators. LDL particles enter the subendothelial space by traversing dysfunctional endothelium. Foam cells coalesce to form fatty streaks, and as lipid and cellular debris increase in volume, an atheromatous plaque forms with a lipid core. As larger amounts of cellular debris accumulate that are no longer cleared by phagocytic macrophages, a necrotic core forms. *Upper left quadrant*. A mature atherosclerotic plaque can be rendered unstable by bleeding into the base of the plaque via disrupted vasa vasora coursing through the tunica adventitia. The sudden increase in blood volume at the base of the plaque raises intra-plaque pressure and can induce plaque rupture, exposing collagen and releasing adenosine 5′-diphosphate and calcium, all of which activate platelets, leading to the formation of overlying thrombus and arterial luminal occlusion. The surface of the plaque may be more prone to rupture because surface matrix proteins have been degraded by matrix metalloproteinases (MMP). *Lower left quadrant*. LDL particles can be oxidized by reactive oxygen species (ROS: superoxide anion, peroxynitrite, hydrogen peroxide) produced by myeloperoxidase (MPO) and a variety of lipoxygenases. Oxidized LDL particles are scavenged by macrophages. Lipoprotein-associated phospholipase A2 (LpPLA2) hydrolyzes phospholipids into lecithin and a free fatty acid, both of which promote inflammation. Scavenged cholesterol can be stored as either pools of oxysterol or as cholesterol crystals. Cholesterol crystals can pierce through plaque surface area and promote platelet activation and thrombus formation. As atherosclerotic plaque becomes more inflamed and less stable, it can rupture, also potentiating platelet activation and thrombus formation. (With permission from Dr. Thomas Dayspring)

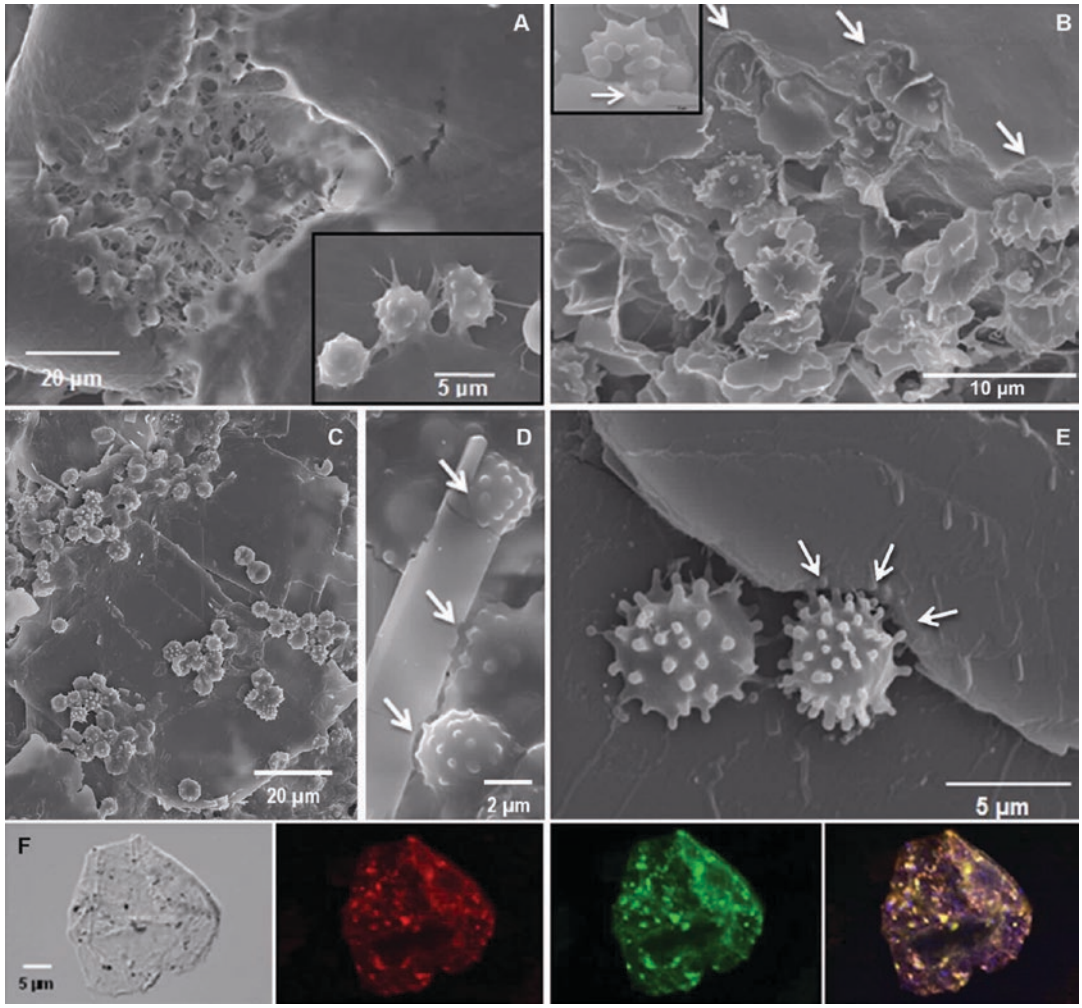

Fig. 2.12 Cholesterol crystals and atherosclerotic disease. Macrophages from coronary aspirates appear to be eroding cholesterol crystals. (**a**–**e**) Scanning electron micrographs demonstrate macrophages engaging cholesterol crystals with notched crystal matrix (arrows). Inserts demonstrate macrophage gummy attachment to the crystal edges and etching (arrow) of the crystal surface. (**f**) Confocal fluorescence microscopy de1monstrates cholesterol aggregates suggestive of crystalline cholesterol (yellow-green particles stained with cholesteryl Bodipy-C12) within the cytoplasm of aspirated macrophages. The orange-red fluorescence is a specific marker for macrophages. Cholesterol deposits can be detected in the cytoplasm using differential interference contrast (shown in gray) and fluorescence microscopy (red, green, and composite image). The unstained control did not exhibit fluorescence (not shown). (From Abela et al. [172]. With permission from Elsevier)

nucleotide-binding domain leucine-rich repeat-containing family, pyrin domain-containing 3 inflammasome that in turn stimulates IL-1β production [170]. Clusters of cholesterol crystals can also be released from culprit plaques during an acute MI and correlate with increased arterial narrowing and reduced reflow subsequent to percutaneous coronary intervention [172].

Conclusions

1. Atherosclerosis is an arterial disease of enormous complexity, whose trajectory is determined by a plethora of genetic and environmental determinants.
2. Atherogenesis encompasses every histologic component in all layers of the arterial

wall (endothelium, intima, media, and adventitia).

3. Atherosclerosis is not simply a process of passive accumulation of apo B-containing lipoproteins in the subendothelial space over time; lipid accumulation and plaque formation are the end result of a highly orchestrated and tightly synchronized network of interlacing pathways involving inflammation, oxidation, and reorganization.
4. Endothelial dysfunction is an early transition point in atherogenesis and is a response to the toxic effects of dyslipidemia, hypertension, smoke exposure, impaired glycemic control, insulin resistance, and a myriad of other risk factors.
5. Endothelial dysfunction is associated with adhesion molecule expression, reduced nitric oxide production, a more thrombogenic surface, and reduced gap junction function.
6. An atherogenic milieu is characterized by heightened oxidative and inflammatory tone; an influx of inflammatory white blood cells, alterations in intravascular cell migration patterns, and an expansion of the vasa vasorum.
7. As plaque evolved and becomes more complex, it can become unstable due to a variety of architectural alterations, among them most notably thinning of the fibrous cap, surface erosions, and leaky adventitial vasa vasora.
8. Acute coronary syndromes are the result of plaque rupture with the formation of overlying thrombus, leading to arterial luminal obstruction, ischemia, and tissue necrosis.
9. There is evidence that at least some atherosclerotic plaques can be reversed, though it is not clear if the arterial wall can be "healed" once a plaque has formed.
10. Despite the fact that we still have much to learn about this disease, using such pharmacologic agents as statins, inhibitors of the renin-angiotensin-aldosterone axis, aspirin, eicosapentaenoic acid, P2Y12 inhibitors, and some antiglycemic agents have all been shown to beneficially impact the course of this dreaded and highly prevalent disease and, most importantly, reduce risk for acute cardiovascular events.

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