Chapter 11

Vascular Smooth Muscle Cells and the Comparative Pathology of Atherosclerosis

Hafidh I. Al-Sadi

Additional information is available at the end of the chapter

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1. Introduction

Atherosclerosis (ATH) is a condition characterized by thickening of the arterial intima as a result of the accumulation of fatty materials (mainly cholesterol and cholesterol esters). Lesions of the disease (atheromas or atherosclerotic plaques) have three distinct components, (1) the atheroma which is the nodular accumulation of a soft, flaky yellowish material at the center of the large plaques, composed of macrophages nearest the lumen of the artery, (2) underlying accumulations of cholesterol crystals, and (3) calcification of the older or more advanced lesions. In human beings, ATH is major cause of adult mortality in the developed world [1]. In animals, ATH occurs rarely, and only infrequently leads to a clinical disease such as infarction of the heart and brain. Several studies have indicated that the pig, rabbit, and chicken are susceptible to experimental induction of the disease through feeding of a high- cholesterol diet and that the dog, cat, cow, goat, and rat are resistant. Naturally – occurring disease has been reported in aged pigs and birds and in dogs suffering from hypothyroidism that accompanies hypercholesterolemia [2].

The smooth muscle cells (SMCs) constitute the predominant cellular element of the vascular media, cause vasoconstriction or dilation in response to physiological or pharmacological stimuli, synthesize extracellular matrix (collagen, elastin and proteoglycans); elaborate growth factors and cytokines; and migrate to the intima and proliferate after vascular injury. These activities of SMCs are important in both normal vascular repair and pathological processes such as ATH. Physiological regulation of the migratory and proliferative activities of the SMCs is regulated by growth promoters and inhibitors. Among the promoters are the platelet – derived growth factor derived from platelets (and endothelial cells and macrophages), basic fibroblast growth factor, and interleukin 1. Inhibitors include heparin sulfates, nitric oxide, interferon γ, and transforming growth factor β.

Injury of the wall of blood vessels stimulates SMCs growth by disrupting the physiological balance between inhibition and stimulation. Repair of the injured vascular wall constitutes a

physiologic healing response with the formation of a neointima, in which SMCs: (1) migrate from the media to the intima, (2) proliferate as intimal SMCs, and (3) elaborate extracellular matrix. During the healing process, the SMCs in the intima lose the capacity to contract and gain the capacity to divide. Within the intima, the SMCs may return to the nonproliferative state when either the overlying endothelial layer is re – established after acute injury or the chronic inflammation ceases. However, intimal thickening occurs when the healing response is exaggerated and this can cause stenosis or occlusion of small and medium sized blood vessels.

2. Functional roles of VSMC in atherosclerosis

Functional roles of VSMC in atherosclerosis include: (1) phenotypic switching (from quiescent "contractile "phenotype to active "synthetic "state; (2) extracellular matrix (ECM) deposition; (3) proliferation; (4) migration; (5) inflammatory gene expression, (6) oxidant stress; and (7) monocyte retention (monocyte – VSMC binding) (Figure 1).

2.1. Phenotypic switching

Several studies demonstrated that the vascular smooth muscle cells have phenotypes that differ in the media and atherosclerotic lesions, and that phenotypic switching of SMCs plays a central role in atherosclerosis according to Ross's hypothesis [3, 4]. It has been proposed that before migration from the media into the intima, a transition of the SMCs phenotype is required [5]. In the media, the SMCs have a contractile phenotype that enables them to regulate the vascular tone. When these cells proliferate they acquire a synthetic phenotype. During the proliferative state the SMC requires extensive changes in gene expression and protein synthesis [6].

A major challenge in understanding differentiation of the SMCs is their ability to appear as a wide range of different phenotypes at different stages of development, and even in adult organism the cells are not terminally differentiated and are capable of major changes in their phenotype in response to changes in their local environment [7, 8, 9]. During early stages of vasculogensis SMCs are highly migratory and undergo rapid cell proliferation. Many studies indicated that there is a remarkable amount of movement of SMCs and SMC progenitor cells as part of the complex morphogenic events that leads to the formation of the cardiovascular system [10, 11]. During vascular development, SMCs also show high rate of synthesis of extracellular matrix components including collagen, elastin, proteoglycans, cadherins, and integrins that share the formation of blood vessel mass. In this stage of development, SMCs form abundant gap junctions with endothelial cells, and the process of investment of endothelial tube with SMCs or pericytes is necessary for vascular maturation and vessel remodeling [12]. In comparison, the SMCs in adult blood vessels show very low rate of proliferation turnover, are largely nonmigratory, show a very low rate of synthesis of extracellular matrix components, and are committed only to carry their contractile function [9]. Mature fully differentiated SMCs express a repertoire of appropriate receptors, ion channels, signal transudation molecules, calcium regulatory protein, and contractile protein

[7]. Following vascular injury the "contractile" SMCs are capable of undergoing transient modification of this phenotype to a highly "synthetic ' phenotype, and they play an important role in the repair of vascular injury. However, that repair of vascular injury is carried out principally (or exclusively) by reversible phenotype modulation of preexisting SMCs is a matter of controversy. Many studies proposed two alternative mechanisms although in reality none is mutually exclusive. In the first one there is evidence that the circulating bone marrow derived SMC progenitor cells play a major role in the repair of vascular injury [13, 14, 15]. The second proposed mechanism is supported by the evidence that SMC populations within blood vessels are extremely heterogeneous with resident stable populations of preexisting SMCs that are phenotypically distinct from the classical definition of a contractile SMCs [16, 17] and that these cells accomplish the injury repair [9].

Some recent studies have provided evidence that circulating cells, presumably derived from bone marrow, can contribute to neointima formation and repair of vascular injury [13, 15, 18]. However, these studies involved very extensive damage to medial SMCs (almost complete destruction of the media and SMCs death), and / or immunologic injury due to genetic mismatch of host and donor tissues following tissue transplantation combined with lack of adequate immunosuppression therapy. However, there is no single study in the severe mechanical injury models have provided strong evidence that bone marrow cells within lesions express definitive SMC markers such as smooth muscle (SM) myosin heavy chain (MHC) and smoothelin. In addition no studies have been made on the possibility of fusion of circulating progenitor cells with resident SMCs [9].

A number of studies are available demonstrating that there are heterogeneities between SMCs within the blood vessel with retention of a resident stable population of cells that have a "synthetic phenotype" [17, 19]. In the study of [19] a panel of antibodies specific for different markers of SMC differentiation including SMC α – actin, SM MHC, calponin, desmin, and meta – vinculin were used to perform immunofluorescence labeling studies on cryosections of adult and fetal bovine main pulmonary arteries. The authors performed also Western analyses of these marker genes in the three different layers of the adult bovine pulmonary artery. These authors reported the presence of four distinct populations or clusters of MSCs based on morphology, cell orientation, pattern of elastic lamellae, and immunostaining patterns and proposed that these populations may represent unique lineages performing different functions within the arterial media, and respond in different ways to stimuli. Although strong evidence exists for the presence of heterogenous populations of SMCs in vivo no studies were done to demonstrate that these represent distinct stable SMC lineages that play a preferential role in carrying out repair of vascular injury in vivo. The studies of Clowes and co-workers [20, 21, 22] would seem to refute such a possibility in that they showed SMC growth fractions (the fraction of medial SMC at time O that leave Go and reenter the cell cycle) of up to 60% following balloon injury of the rat carotid artery, indicating that the majority of SMCs within the media possess the ability to reenter the cell cycle and contribute to repair of vascular injury in adult animals. Thus the preexisting "subpopulation "of SMC capable of phenotypic switching is much greater than frequencies reported by [19] and represent a large fraction of SMCs in the vessel wall. [23] using the generation of

complex SMC ancestor tables for the entire SMC population within the thoracic and abdominal aorta based on pulse – chase labeling with [^{3}H] thymidine in hypercholesteremic swine models of atherosclerosis provided evidence that supports the previous findings. They found that the intimal lesions were polyclonal and derived from multiple histologically discrete medial SMCs that initiated DNA replication and subsequently underwent several rounds of DNA replication. These findings are in discrepancy with a model in which only a small fraction of medial SMCs contribute to lesion formation.

Results of another study [24] reported that distinct populations of rat cultured SMCs (adult and embryonic) when implanted into a rat carotid artery in vivo retained some phenotypic differences. These findings suggest that there is considerable stability in the phenotype of these cells. However, it is possible that the stable epigenetic reprogramming of these cells was a function of their extensive growth in culture. Additionally, since large number of cells were transplanted, it is possible that the transplanted cells could created their own "microenvironmental domain or milieu "and that autocrine and paracrine effects contributed to the retention of phenotypic differences. [25] suggested the existence of a subpopulation of terminally differentiated SMC that is incapable of cell cycle reentery. Evidence for this suggestion was based on studies showing failure of a subpopulation of SMC derived from dog aorta to proliferate in culture. However, this finding may repersent the lack of appropriate culture reagents and / or conditions necessary to support growth of these cells.

In summary, it seems that the principal source of SMCs responsible for repair of vascular injury under "normal "circumstances are the preexisting SMCs that undergo transient and reversible phenotypic modulation. However, circulating bone marrow cells, cells derived from the adventitia, and / or preexisting subpopulations of phenotypically modified SMC can participate to some extent as well. The role of each of these different populations differs according to the nature of the vascular injury or the disease state.

2.2. ECM deposition

ECM comprises > 50 % of the atherosclerotic lesion and it consists of a mixture of vastly different macromolecules including collagen, elastin, glycoproteins, fibronectin, laminin, vitronectin, and thrombospondin. These matrix proteins are produced largely by the activated VSMCs [26, 27] and confers tensile strength and viscoelasticity to the arterial wall. Each of these ECM components possesses unique structural properties that determine its own roles during the development of atherosclerotic plaques. In addition to its role in supporting the plaque, ECM participates in many key events such as cell migration and proliferation, lipoprotein retention and thrombosis.

Matrix metalloproteinases (MMPs) are endopeptidases produced by SMCs and macrophages and they contribute significantly to the degradation and remodeling of the plaque extracellular matrix [29]. MMps that are induced to be expressed by environmental factors present within the lesion can actively modify the matrix in which SMCs reside and actively contribute to further phenotypic switching of the SMC. SMC phenotype is prone to

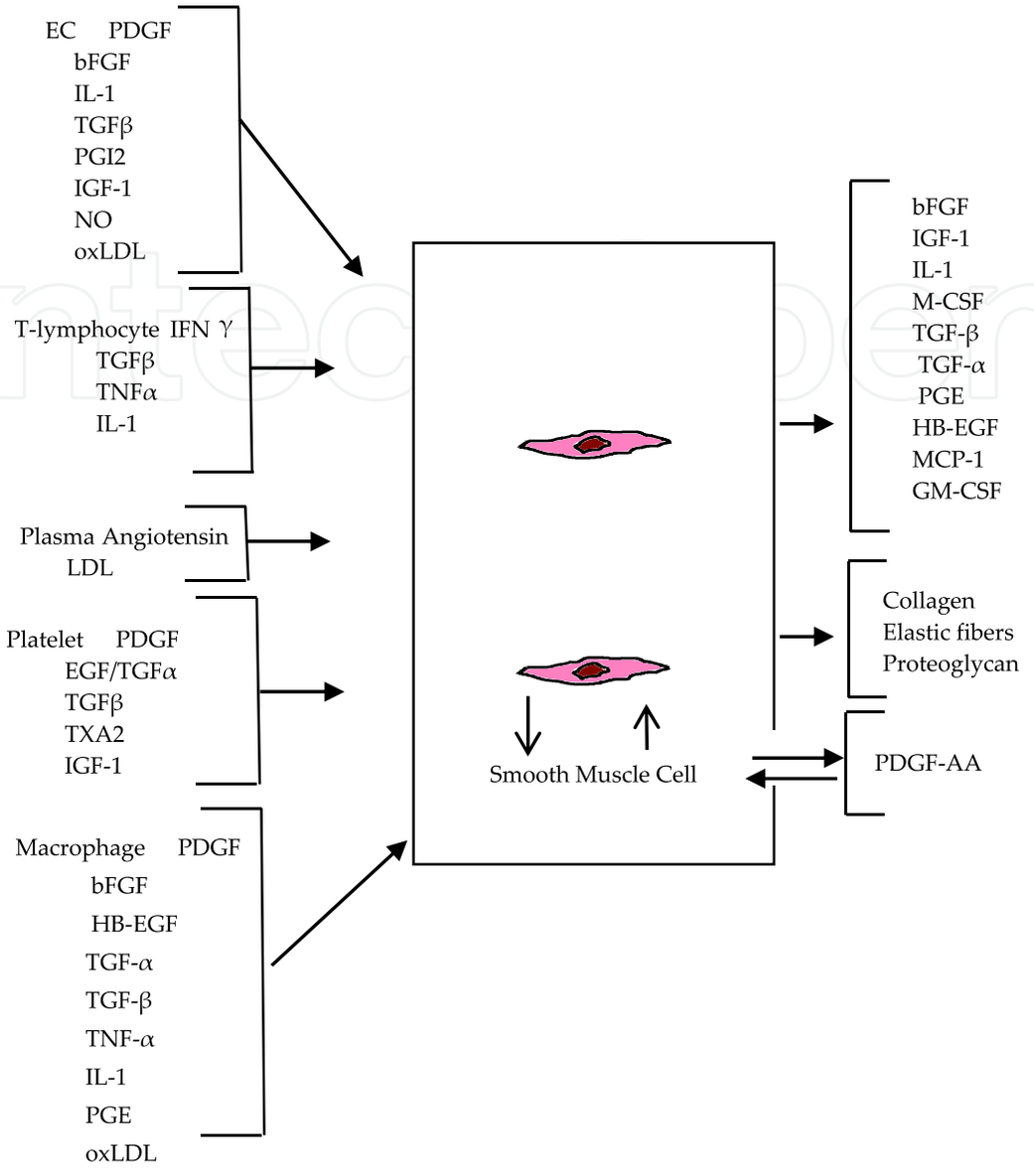

Figure 1. Redrawn and modified from Ross [28].

modification by a number of factors including PDGF [30], TGF β [31], intric oxide [32], and reactive oxygen species [33]. These factors have been demonstrated to modify MMP production in cultured SMCs, although information concerning the mechanisms and factors that control expression of MMPs in vivo are still lacking. A major determinant of plaque stability is the existence of a balance between production of matrix degrading MMPs, the inhibitors MMPs or tissue inhibitor of metalloproteinase (TIMPs), and matrix production by SMCs [34, 35]. In normal arteries of human and laboratory animals, MMP – 2 (72 – Kda gelatinase) and TIMp – 1 and TIMP – 2 are constitutively expressed at levels creating a stable balance between endogenous matrix proliferation and matrix degradation m and a normal MMP – to – TIMP ratio [29]. In the developing atherosclerotic lesion, this ratio is tipped towards MMPs as described in part by an increase in MMP – 3 (stromelysin) and MMP – 9 (92 – Dka gelatinase), and increased MMP – to – TIMP ratio [29]. It would seem that MMP overexpression is important for the migration and formation of a SMC – rich

fibrous plaque and subsequent plaque stabilization. Galis *et al.* [36] presented evidence that MMP – 3 and MMP – 9 could be also expressed by SMCs in the shoulder region of human atherosclerotic plaques. [37] found a correlation between decrease of SM – 1 and SM – 2 marker expression and increase in PDGF and MIM – 3/ 9 expression in the rabbit neointima. Knoockout gene studies for MMP – 9 have demonstrated decreased intimal SMC hyperplasia and reduced late lumen loss in the mouse carotid artery flow cessation model [38] and in the carotid wire injury model [39]. During the natural progression of atherosclerosis in APOE – 1- mice, differential expression of MMP – 9 increases with time in lesional areas versus nonlesion areas [40]. Lemaitere *et al.* [35] showed that in TIMP – 1 / - mice crossed to APOE – 1- mice there was increased aortic medial ruptures compared with control mice after 10 weeks of Western diet feeding, indicating imbalance between MMP expression and TIMP expression can lead to lesion instability.

2.3. SMC proliferation

Immediately following activation by the injury, growth factor, or cytokine, the SMC undergoes phenotypic change that leads to a migratory and secretory cell that migrates into the neointima [41]. Stimulation by growth factor or cytokine causes the SMCs to proliferate and secrete matrix proteins and enzymes. Whereas the complex atherosclerotic lesions contain a mixed type of cells including SMCs, lipid laeden macrophages, and lymphocytes, the vascular SMCs are the dominant cellular component of the de novo and in-stent restenotic lesions. Results of both experimental and randomized clinical trials have shown that significant retardation of in-sent stenosis could be achieved through the delivery of inhibitors of inflammation and of SMCs proliferation such as sirolimus and paclitaxel to the site of intervention on drug – eluting stents [42, 43].

Proliferative phenotype of vascular SMCs in both physiological and pathological events involve critical changes in the gene expression patterns of the proliferating cells. Growth-promoting factors such as angiotensin 11 or oxidized low density lipoprotein stimulate the vascular SMCs and lead to increased expression of genes coding for iron transporters, extracellular matrix components, cell – cell adhesion molecules, cytoskeletal proteins, transcription factors and cell cycle regulatory proteins [44, 45]. Comparison between the healthy vascular SMCs and vascular SMCs isolated from various disease conditions, such as primary atherosclerosis and in – stent stenosis reveals similar changes in gene expression patterns [46].

Changes in gene expression programs are influenced largely by transcriptional events, but the contribution of posttranscriptional events (such as mRNA processing, transport, turnover, and translation) is becoming increasingly recognized [47]. One of the most important of these events, stability of regulated mRNA critically contributes to the implementation of gene expression patterns during the cellular response to mitogens, immunological triggers, stress, and differentiation agents [48, 49]. A growing list of proteins central to the execution of such responses (P21, Hsp70, MnsoD, catalase, Cdc25, cyclin A, cyclin B1, c-Fos, c-Jun, c-Myc, Egr-1, etc) are encoded by labile mRNA, which have tightly regulated half – lives [47].

Among the well – studied determinants of transcript stability are U – rich or A + U rich elements (collectively termed AREs) generally present in the 3' -untranslated regions of labile mRNA [50]. Several RNA – binding proteins have been found to bind to AREs and leads to transcript decay and they include BRF1, AUF1, and KSRP [51, 52, 53]. HuR, a member of the Hu / ELAV protein family binds to AREs and instead promote transcript stabilization. HuR stabilizes many mRNAs that encode growth factors, cell division proteins, and cytokines. An important role of HuR during the response to immune factors and proliferate signals has been described in many cell systems [54, 55, 56, 57, 58].

HuR was found to regulate cell proliferation in a mouse model of skeletal muscle development and regeneration, purportedly through its effect on the expression of proteins governing cell growth and differentiation [59, 60]. Pullmann, Jr. *et al.* [47] found that treatment of hVSMCs with platelet - derived growth factor increased HuR levels in the cytoplasm, thereby influencing the expression of metabolic, proliferative, and structural genes. In addition, knockdown of HuR expression by using RNA interference was reported to cause a reduction of hVSMC proliferation, both basally and following platelet – derived growth factor treatment. These authors postulated that HuR contributes to regulating hVSMC growth and homeostasis in pathologies associated with VSMC proliferation [47]. In other studies, HuR was found to increase the rate of proliferation of human diploid fibroblasts, shortened the cell division time of colon cancer cells, and accelerated the development of tumors in nude mice [55, 56, 61]. The stimulatory effect of HuR on cell proliferation is proposed to be though increasing the stability of several mRNAs, including those that encode cyclin A, cyclin B1, c- Fos, c – Myc, and cyclin D1, thereby enhancing the expression of the corresponding proteins,. which promote progression through the cell division cycle [62]. Pullmann, Jr. et al. [47] have identified the cDk2 mRNA as a novel target of HuR. This is an interesting finding in view of the regulatory influence of cdk2 and cyclin A on rat carotid artery VSMC proliferation. It has been proposed that HuR may be a contributing factor to smooth muscle cell and neointima proliferation and consequently to atherosclerosis [47].

In summary, the migratory and proliferative activities of VSMCs are regulated by a balance between growth promoters such as platelet derived growth factors (PGF), endthelin – 1 (ET – 1), thrombin, fibroblast growth factor (FGF), interleukin -1 (IL - 1) and inhibtors such as, heparin sulfates, intric oxide (NO), transforming growth factor (TGF) – beta. A role has been suggested for matrix metallo – proteinases (MMPs) could catalyze and remove the basement membrane around VSMC and facilitate contacts with the interstitial matrix. VSMCs are stimulated to proliferate and migrate by some kinds of cytokines, growth factors, and angiotensin II (Ang – II). Apoptosis, proliferation and migration of VSMCs are essential features of the pathogenesis of atherosclerosis and plaque rupture. Rupture of the plaque is associated with increased number of fibrous cap macrophages, increased VSMC apoptosis and reduced fibrous cap VSMCs. Within the plaques, the VSMCs are the only cells capable of synthesizing structurally important collagen isoforms, and the apoptosis of VSMC might promote plaque rupture [63].

2.4. SMC migration

Agents that induce VSMC migration include growth factors (Angiotensin II, PDGF, bFGF, HB – EGF, IGF – 1, VEGF, Thrombin), cytokines / chemokines (IL – 1 β,. IL – 6, TL -6, TFβ 1, TNF – alpha, MCP – 1), extracellular matrix components (collagen 1, IV , collagen VIII, fibronectin, hyaluronan, laminin, osteopontin thrombospondin, vitronectin), bioactive lipids (LPA, Hydroxyeicosatrienoic acids [12 or 15 (s) – HETE], diabetogenic agents [high glucose (25 mmol / L)], advanced glycation end products (AGEs), RAGE ligands S 100 B, and other molecules (ATP, UTP, norepinephrine, histamine, and serotonin) [64]. Angiotensin II – induces VSMC migration through P38 – MAPK activated c – Src through two distinct but redundant pathways, one via Syk, and other via EGFR transactivation through ERK 1/2 and partially through p38 MAPK [64]. Receptor for advanced glycation end products (RAGE) ligands induce inflammatory genes and VSMC migration via Src kinase [64]. Additionally, RAGE ligand (S100B) activates Src and MAP kinases in VSMCs [64]. In diabetic db / db mice, it has been found that there was enhancement of RAGE expression, Src activation, and migration in VSMCs [64].

2.5. Inflammatory gene expression

The atherogenic cytokines that are released from SMCs, endothelial cells (EC), macrophages, T lymphocytes, and B lymphocytes include IFN (released from SMC, macrophages, T lymphocytes, and causes increased SMC migration and proliferation, increased ECM remodeling, and increased adhesion molecule expression), IL – 1 (released from SMCs, EC, macrophages, and T and B lymphocytes and increases SMC migration and proliferation, monocyte accumulation, and adhesion molecule expression) , IL – 18 (released from SMC, EC, and macrophages, and causes increased adhesion molecule expression, increased SMC accumulation, and ECM remodeling), MCP – 1 (produced by the SMC, EC, macrophages, and T lymphocytes, and increases recruitment of monocytes , SMC migration and ECM synthesis and remodeling), PDGF – BB (produced by the SMC, EC, and macrophages, and increases SMC migration and increases SMC migration anf proliferation), and TGFβ (elaborated by SMC, EC, macrophages, and proliferation and increases SMC migration and proliferation, and ECM synthesis) [64]. Cytokines that are involved in atherogenesis include TNF – α, IL – 1, IL – 6, IL – 8, IL – 12, IL – 15. IL – 18, IL 32, and MCP – 1 produced by macrophages under the influence of resisting leptin, and adiponectin elaborated from adipose tissue. Cytokines released from macrophages stimulate SMC to produce the cytokines TNF – α, IL – 1, IL – 6, and IFN – (?). Those same cytokines also activate the EC to prduce VCAM – 1, ICAM – 1, E – selectin, P – selectin, IL – 1, IL – 6, IL – 8, IL – 18, IL PB, and MCP – 1. Other cytokines also produced by Tho (IL – 2, IL – 3), Th 1 (IL – 2, INF, IL – 17) Th 2 (IL – 4, IL – 5 < IL – 6, IL – 10, IL – 13), Treg (IL – 10, TGFβ), NKT cells (IFN), and macrophages (IL – 10, TGFβ, IL – lra, IL – 18BP) [64].

2.6. Oxidative stress

An important feature of the pathological porcess in atherogenesis is an increased generation of reactive oxygen species (ROS). All components of the atherosclerotic lesion has been

shown to increase production of ROS mainly superoxide anion ($O2^-$) [26, 65]. The ROS are produced by the VSMCs, endothelial cells, fibroblasts, and infiltrating leukocytes [66]. These ROS affect gene transcription, damages DNA, and increases production of inflammatory transcription factors [67]. Oxidation of LDL and scavenging of endothelium – derived NO are the best studied effects [26].

The mechanism of oxidative modification of LDL is unknown but there is always oxidized LDL in atherosclerotic lesions. Experimental studies indicated that the level of oxidized LDL, as measured by autoantibody titers, is reflective of the atherosclerotic burden [68]. Many of the atherogenic processes are induced by the oxidized LDL and these include transcription of proatherogenic genes, production of matrix metalloproteinases and tissue factor, antagonism of endothelial cell production of NO, and promotion of VSMC apoptosis [69]. The increased production of superoxide anion rapidly reacts with NO to produce peroxynitrite, a potent oxidant [70]. It has been stated that scavenging of NO increases inflammation, platelet activation, and vasoconstriction [26].

Studies of antioxidant vitamins, including the Guppo Italiano per 10 Studio della Sopravvivenza nell' Infarto myocradico (GISSI) Prevention Trial, the Heart Outcomes prevention Evalution Study (HOPE), and the Heart protection Study (HPS), did not show any reduction in clinical events with antioxidant vitamin E therapy [71, 72]. However, in these trials there were limitations that precluded the adequate test of the hypothesis ([73]. One of these limitations is that the rate constant for reaction of vitamin E or C with superoxide anion is much slower than for superoxide anion with NO or endogenous antioxidant enzymes [74]. Oral intake only slightly increases the levels of vitamin in plasma and tissue, and this may not affects the events in the vascular wall, where it is not concentrated. Vitamin E contributes little in the form of antioxidant protection in the cytoplasm, nucleus, or interstitial space since it is concentrated in the lipid bilayers. Additionally, a delay in treatment may have abolished its effect on the development of lesions and with little effect on plaque rupture and clinical events. However, some other therapeutic studies in the same trials (e.g. HOPE and HPS) indicated significant benefit [67, 75]. Of equal importance is that conventional antipletlet therapy has antioxidant effects as it has the ability to limit ROS production by activated platelets. The fact that oxidative stress plays important role in the pathogenesis of atherosclerosis makes clear that the limitations of current therapies should not conclude therapeutic interest in this area but stimulates studies into new ways of treatment.

2.7. Monocyte – VSMC binding

Monocytes in circulation adhere and migrate across the endothelium in response to atherogenic stimuli. Initially, these processes may be reversible, whereas subsequent accumulation and retention of monocytes – macrophages into the intima become a central pathogenic process in atherogenesis [76]. The mechanisms by which the monocytes are retained in the subendothelium and the role of VSMCs in this process are not known. Adhesive reactions between marginated monocytes and VSMCs have been proposed to contribute to monocyte- macrophage retention in the intima. Evidence for the possible

interaction between VSMCs and the monocytes comes from the fact that VSMC express adhesion molecules within the atherosclerotic lesions but not in normal vessels [77]. In addition, a highly significant association was demonstrated between VSMC vascular cell adhesion molecule (VCAM) – 1 expression and the content of the intimal macrophage [78, 79]. Furthermore, a significant focal expression of intercellular adhesion molecule (ICAM) – 1 on VSMC in regions prone to atherosclerosis was found preceding mononuclear cell infiltration in man, which indicates a causative role in lesion development [80]. It has been shown that cell – to – cell interactions between monocytes and VSMC enhanced the procoagulant activity of monocytes and increased the production in both cell types of atherosclerosis – related materials such as metalloproteinase – 1 [81]. These findings indicated that the VSMCs and monocytes – macrophages are not merely neighbors in the vessel wall but that VSMC – monocyte interactions constitute additional signals in the pathogenesis of atherosclerosis. However, the cellular, molecular, and signal transudation mechanisms need to be elucidated.

A well-known fact is that angiotensin II (ANG II) and platelet – derived growth factor (PDGF) – BB have a significant role in vascular remodeling and atherosclerosis [82]. They are capable of inducing VSMC migration, hypertrophy, and proliferation [82, 83]. Cai *et al.* [84] investigated the effects of angiotensin II (ANG II) and PDGF – BB on VSMC – monocyte interactions. They found that treatment of human aortic VSMC (HVMC) with ANG II or PDGF – BB significantly increased binding to human monocytic THP-1 cells and to peripheral blood monocytes. This was inhibited by antibodies to monocyte β1 – and β2 – integrins. Attenuation of the binding was also achieved through blocking of VSMC arachidonic acid (AA) metabolism by inhibitors of 12 / 15 – lipoxygenase (12 / 15 – LO) or cyclooxygenase -2 (COX - 2). On the other hand, enhancement of binding was obtained by overexpression of 12 / 15 – LO or COX – 2. Binding was also enhanced by direct treatment of HVSMC with AA or its metabolites. Additionally, VSMC derived from 12 / 15 – LO knockout mice showed reduced binding to mouse monocytic cells in comparison with genetic control mice. Using specific signal transudation inhibitors, Cai *et al.* [84] showed the involvement of Src, phosphoinositide 3 – kinase, and MAPKs in ANG 11- or PDGF – BB induced binding. These authors also found that after coculture with HVSMC, THP – 1 cells surface expression of the scavenger receptor CD36 was increased. In conclusion, results of the work of Cai *et al.* [84] indicated that the growth factors may play additional roles in atherosclerosis by increasing monocyte binding to VSMC via AA metabolism and key signaling pathways. This process can lead to monocyte subendothelial retention, CD36 expression, and foam cell formation.

3. VSMCs in the pathogenesis of atherosclerosis

3.1. Humans

Three major types of cells that are commonly seen in the atherosclerotic lesions are the SMCs (which dominates the fibrous cap), macrophages (inflammatory cells) that infiltrate around the necrotic core, and the lymphocytes (intracellular and intercellular lipid) which have been mainly ascribed to the fibrous cap [85, 86]. A very complex interplay exists

between these cells in the various developmental stages of the atherosclerotic lesion. This complex interrelation is further complicated by a number of risk factors that contribute to the clinical manifestation of the disease, and they include the abnormal vasomotor function, the thrombogenicity of the blood vessel wall, the state of activation of the coagulation cascade, the fibrinolytic system, SMC migration and proliferation, and inflammation process [76, 86, 85]. However, the exact cause of development of the atherosclerosis is not completely understood. Similarly much more information is needed concerning regulation of the key role of SMCs in vascular injury repair and in the development and / or progression of atherosclerosis. Finally, additional work is needed to clarify the specific contributions of the SMC versus other cell types within the lesion, such as macrophages and endothelial cells, to the end-stage clinical sequelae of atherosclerosis including plaque rupture, thrombosis, infarction, vasospasm, myocardial ischemia, and death.

3.2. Birds

Various avian species such as pigeons [87, 88], turkeys [89], and chickens [90] have been shown to be the convenient experimental animals for induction of atherosclerosis. Shih *et al.* [91] mentioned that the Japanese quail is an ideal laboratory animal for long – term experiment because of its small size, short life cycle, and low feed consumption. Atherosclerosis was conventionally induced in various experimental animals through feeding of cholesterol and fat or oil [88, 89, 90]. Oku *et al.* [92] demonstrated that dietary feeding of 2 % cholesterol and 15 % corn oil for 3 months can induce typical atherosclerotic lesions more frequently in the ascending aorta and its large branches than in abdominal aorta in Japanese quails. This finding is comparable to that reported by Toda *et al.* [93] in the chickens. Atherosclerosis was also induced in quails by feeding them with 2 % cholesterol and 0.5 % cholic acid for 15 weeks [94]. Morrissey and Danalbson [95] demonstrated that atherosclerosis could be induced by feeding quails with 1 % cholesterol and 10 % fat for 10 weeks. Wexler [96] reported that both the male and female Japanese quails developed spontaneous atherosclerosis at 2 years of age. Jarrold *et al.* [97] and Velleman *et al.* [98] have demonstrated that the cholesterol – induced atherosclerosis (CIA) line of Japanese quails is a valid animal model to study ECM remodeling induced by hypercholesterolemia. Jarrold *et al.* [97] showed that the proteoglycan decorin was localized in the foam cell regions and collagen type I was found to surround the foam cells where decorin accumulated. Velleman *et al.* [99] showed that remodeling of the collagen component of the dorsal aorta extra – cellular matrix during the progression of atherosclerosis in Japanese quails selected for CIA. Toda *et al.* [93] reported that fibroblasts rather than smooth muscle cells are the main cellular component in the development of atherosclerosis in Japanese quails as in chicken. Oku *et al.* [92] suggested that phenotype transformation of intimal cell migrating from the tunica media to play an important role in the initiation and the development of atherosclerosis. Casale *et al*. [100] studied the cellular events of quail atherosclerosis using monoclonal antibodies to alpha-actin and chicken macrophages and effectively identified the presence of SMC and macrophages, respectively, as constituents of the atherosclerotic lesions. The presence of macrophage, as well as SMC proliferation, was ob-

served in early lesions. Although it was not possible to acertain the first cell type to be involved in the initial stages of atherosclerosis, results suggested early intervention of macrophages and SMC. Bavelaar *et al*. [101] studied the possibility that feeding of α-linolenic acid instead of linoleic acid or saturated fatty acids would diminish the degree of atherosclerosis in cholesterol-fed quails. The authors concluded that a differential effect on the development of atherosclerosis of α-linolenic acid, linoleic acid and saturated fatty acids could not be demonstrated.

Fabricant and Fabricant [102] investigated the roles of both Marek's disease herpesvirus (MDV) and dietary cholesterol in atherosclerosis in chickens. The birds were examind 7 months after MDV infection with and without cholesterol feeding for gross and microscopic arterial lesions. Typical lesions of atherosclerosis were observed only in infected normocholesterolemic or hypercholesterolemic birds. They were not detected in uninfected birds even if the birds were hypercholesterolemic. Furthermore, immunization with turkey herpesvirus vaccine or SB – 1 vaccine prevented atherosclerotic lesions. Hajjar *et al*. [103] demonstrated histologically that infection of normocholesterolemic, specific-pathogen-free chickens with Marek's disease herpesvirus (MDV) lead to chronic atherosclerosis like that in humans (Figures 2 and 3).

Spontaneous atherosclerosis is of common occurrence in captive parrots [104]. It occurs in all parrot species but with the highest occurrence in African Grey parrots and Amazons. Old birds are more commonly affected, and the disease has been seen in both males and females. Sudden death is the most common signs, but clinical symptoms include dyspnea, lethargy and nervous sign, such as paresis and collapses. Atherosclerosis is mostly an unexpected finding at necropsy because clinical signs of the condition are seldomly seen and the difficulty associated with diagnosis. In parrots, age and species are determinants of atherosclerosis. Risk factors have been suggested to include an elevated plasma cholesterol level, diet composition, social stress, and inactivity [104]. Frick *et al*. [105] studied the incidence of spontaneous atherosclerosis in 62 African grey parrots (*Psittacus erithacus*) and 35 Amazon parrots (*Amazona spp.*). Incidence of atherosclerosis was 91.9% in African grey parrots and 91.4% in Amazon parrots. According to the missing lymphocytes and macrophages and the absence of invasion and proliferation of SMCs, the authors concluded that the "response-to-injury hypothesis" is inapplicable in parrots.

3.3. Pigs

In recent years an increase occurred in use of pigs as a promising species for morphological, biochemical, and metabolic studies of cardiovascular diseases particularly atherosclerosis [106, 107, 108]. Anatomy and physiology of the cardiovascular system of pigs resemble that of man. Among the similarities are size and distribution of arteries, location of thickened intima in the normal state, blood pressure, heart rate, plasma lipoprotein patterns, and responses to diets rich in fat and cholesterol [108]. Spontaneous atherosclerosis is known to develop in pigs with increased age, and have lipoprotein profiles and metabolism similar to

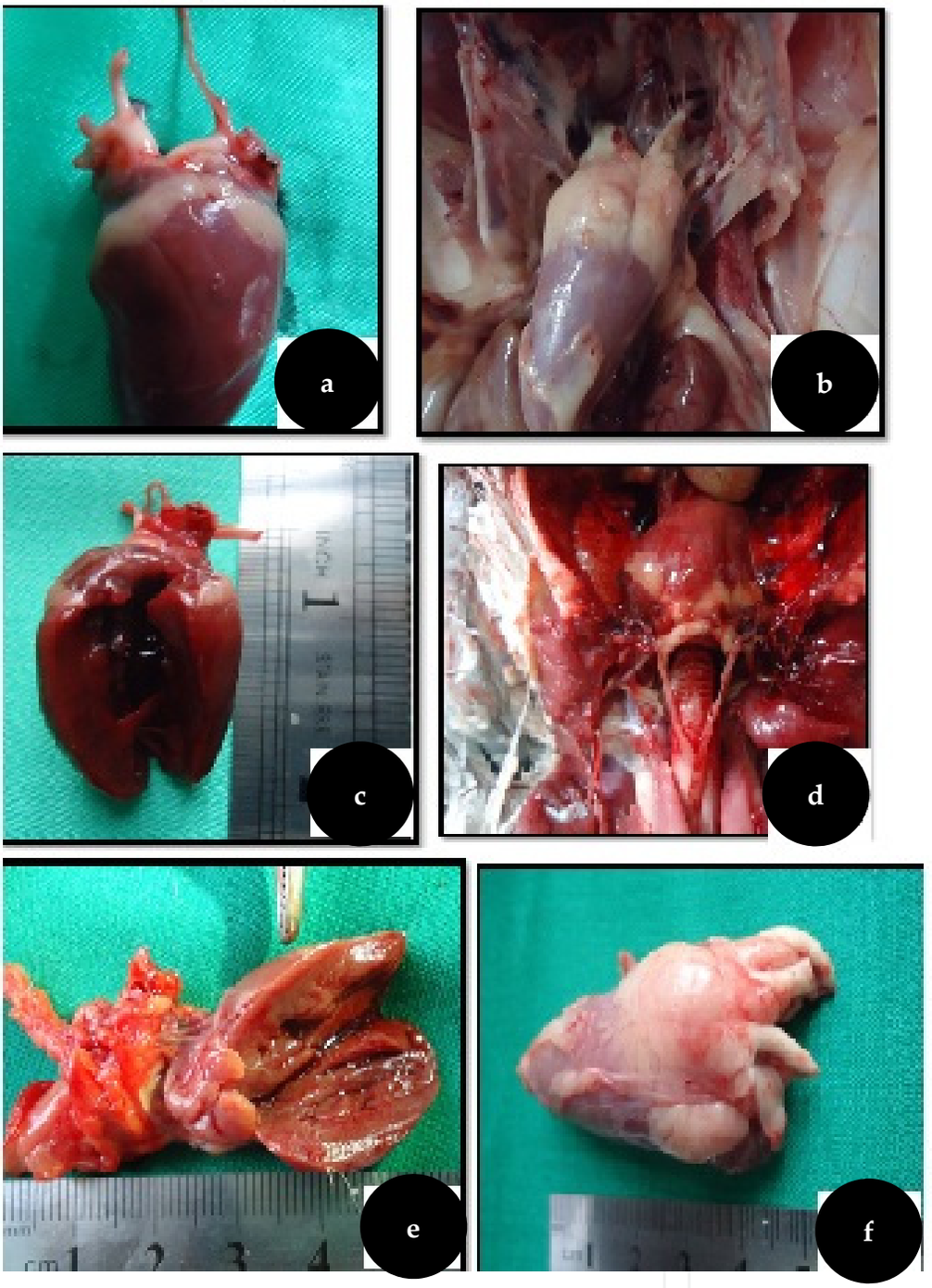

Figure 2. **a.** Heart and major blood vessels of a pigeon (*Columba livia*). Hyperatrophy of the heart and thickening of major blood vessels (arrows) could be visualized. **b.** Thoracic cavity of a pigeon (*Columba livia*). Hyperatrophy of the heart, fat deposition on the heart, and thickened blood vessels originating from the heart (arrows) could be visualized. **c.** Same heart shown in figure 2 following opening the chambers and shows narrowing of chambers as result of myocardial hypertrophy (arrows). **d.** A view of thoracic cavity of a chicken .A rounded hyperatrophied heart (H) with thickened major blood vessels (arrows) could be seen. **e.** Heart and major blood vessels of a chicken following opening of heart to show narrowing of ventricles as result of myocardial hyperatrophy. **f.** Heart and major blood vessels of a New Zealand White rabbit. Note fat deposition on pericardium and thickening of the walls of major blood vessels.

humans [106, 107]. Use of high fat, high cholesterol diets in pigs leads to elevation of total and LDL plasma cholesterol [106]. Several studies have shown the ability to induce aortic lesions in pigs very similar to those seen in human atherosclerosis disease [106].

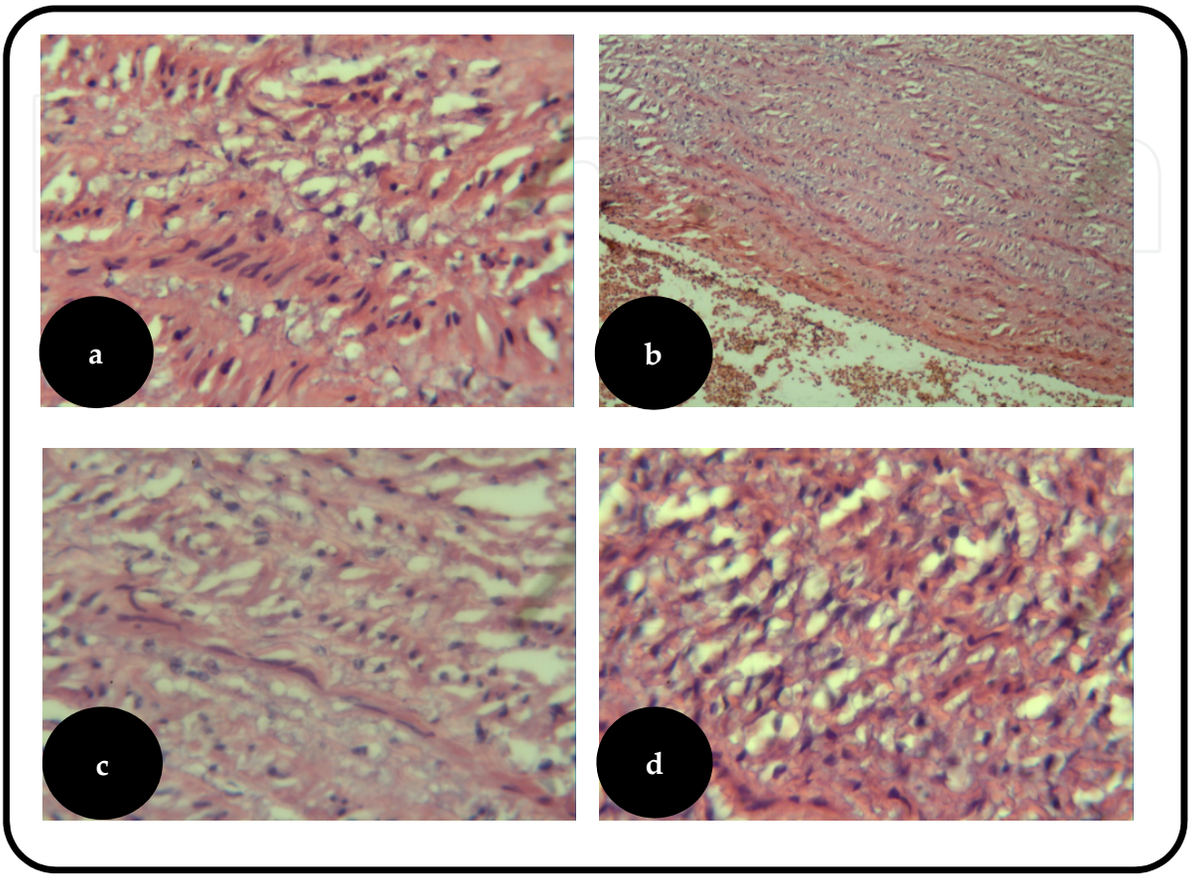

Figure 3. **a.** Microphotography of arterial wall in a pigeon (*Columba livia*) showing lipid accumulation in tunica media . H&E. **b.** Microphotography of arterial wall in a pigeon (*Columba livia*) showing lipid accumulation in tunica media and destruction of some of the elastic lamellae. H&E.
c. Microphotography of arterial wall in a pigeon (*Columba livia*) showing destruction of the elastic lamellae . H&E. **d.** Microphotography of arterial wall in a chicken showing lipid accumulation in the intima. H&E.

3.4. Rabbits

Among animals that have been used as models for experimental atherosclerosis, the rabbit is the only one that has the tendency to exhibit hypercholesterolemia within a few days of administration of a high cholesterol diet [110, 111]. Furthermore, the late stages lesions of human atherosclerosis are similar to those caused in rabbits when a diet low in cholesterol is administered for extended periods [110]. More advanced lesions in the thoracic and abdominal aorta, could be induced in rabbits through a high cholesterol diet can be combined with a single or double balloon injury. Balloon injury enhances the formation of atheromatic lesions and leads to the production of plaques with a lipid core covered by a fibrous cap with a high amount of SMCs. Such lesions are more similar to human atherosclerotic lesions than those produced by feeding rabbits with a high cholesterol diet alone [37, 112].

Hypercholesterolemia that is induced in rabbits through diet is caused by the accumulation of exogenous cholesterol. Rabbits cannot increase the excretion of sterols and this explains their high susceptibility to inducement of atherosclerosis [113]. Consequently, increased quantities of lipoproteins rich in cholesterol esters enter the blood circulation. LDL and β – VLDL are the main transporters of cholesterol in plasma. They remain for prolonged time in blood circulation [113].

In rabbits, morphological features of the lesion could be modified by the percentage of cholesterol added to the diet and the duration of the diet [111, 113]. Diets with a percentage of cholesterol of more than 2 % and given for short duration, cause hypercholesterolemia, and atherosclerotic lesions rich in foam cells originate from macrophages. In contrast of this, a diet with a low cholesterol content, and long duration causes atherosclerosis lesions, which are rich in SMCs and contain cholesterol deposits leading to atherosclerosis lesions more similar to those of humans [113]. More advanced lesions were found to be formed not with continuous but with intermittent atherogenic diets [113]. Additionally, increasing the percentage of cholesterol in the diet to more than 0.15 %, cholesterol esters were detected in the lesion [113]. Spagnoli *et al.* [114] found that the formation of advanced lesion depends on the age of the animal. Thus, old rabbits (3 – 4.5 years old) exhibit fibrotic plaques while young rabbits (4 months old) do not have such advanced lesions (Figure 2-f).

Author details

Hafidh I. Al-Sadi
Comparative Pathology, Department of Pathology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

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