Journal of Biomedical Science (2005) 12:839–853
DOI 10.1007/s11373-005-9024-z

Molecular aspects of atherogenesis: new insights and unsolved questions

Giovanni Maria Puddu[2], Eleonora Cravero[1], Giorgia Arnone[2], Antonio Muscari[1,*] & Paolo Puddu[1]

[1]*Department of Internal Medicine, Cardioangiology, Hepatology, University of Bologna – S. Orsola-Malpighi Hospital, Via Albertoni, 15, 40139, Bologna, Italy;* [2]*Department of Internal Medicine and Aging, S. Orsola-Malpighi Hospital, Bologna, Italy*

Received 7 July 2005; accepted 17 August 2005

Key words: atherogenesis, oxidant stress, G proteins, caveolin, lipoproteins, statins

Summary

The development of atherosclerotic disease results from the interaction between environment and genetic make up. A key factor in atherogenesis is the oxidative modification of lipids, which is involved in the recruitment of mononuclear leukocytes to the arterial intima – a process regulated by several groups of adhesion molecules and cytokines. Activated leukocytes, as well as endothelial mitochondria, can produce reactive oxygen species (ROS) that are associated with endothelial dysfunction, a cause of reduced nitric oxide (NO) bioactivity and further ROS production. Peroxisome proliferator-activated receptors (PPAR) and liver X receptors (LXR) are nuclear receptors significantly involved in the control of lipid metabolism, inflammation and insulin sensitivity. Also, an emerging role has been suggested for G protein coupled receptors and for the small Ras and Rho GTPases in the regulation of the expression of endothelial NO synthase (eNOS) and of tissue factor, which are involved in thrombus formation and modulation of vascular tone. Further, the interactions among eNOS, cholesterol, oxidated LDL and caveola membranes are probably involved in some molecular changes observed in vascular diseases. Despite the relevance of oxidative processes in atherogenesis, anti-oxidants have failed to significantly improve atherosclerosis (ATS) prevention, while statins have proved to be the most successful drugs.

Introduction

Atherosclerotic disease, with its ischemic sequelae, continues to be the principal cause of death and disability in developed countries. The worldwide research concerning causes, pathogenesis and treatment of this disease has become increasingly intensive, resulting in an impressing exponential growth of the knowledge in genetics, molecular biology and pharmacology. It is certainly beyond the scope of this review article to provide a detailed picture of all the research developments in this field. However, some of the most promising advancements in the knowledge on atherogenesis will be outlined, together with their most recent literature references. In particular, the following items will be considered:

(1) Genetic predisposition,
(2) Peroxisome proliferator-activated receptors (PPAR) and liver X receptors (LXR),
(3) Oxidant stress and endothelial cell dysfunction,
(4) Mitochondrial dysfunction,
(5) Effects of lipids and oxidized LDL on intimal cells,

*To whom correspondence should be addressed. Fax +39-051-6362210, E-mail: amuscari@med.unibo.it

(6) Recruitment of mononuclear leucocytes,
(7) Rho and Ras,
(8) Other G proteins in atherogenesis,
(9) Caveolin and cholesterol trafficking,
(10) HDL and atherogenesis,
(11) The mature atherosclerotic lesion and atherothrombosis,
(12) Some therapeutic implications.

Genetic predisposition

Atherosclerosis (ATS) is an inflammatory disease that may persist for several years before clinical manifestations, such as heart attack, stroke and peripheral arterial disease, become evident. In fact, the initiating atherogenetic processes require prolonged exposure to predisposing factors, and most individuals initiate ATS in a very early stage of their life. In most cases the development of atherosclerotic disease results from the interaction between the environment and the genetic make-up. Although the most common forms of ATS are multifactorial, forms with a strong genetic component are also well known. Nevertheless, so far, the role of only a minority of genetic risk factors for ATS has been fully elucidated. In a few cases, the disease is monogenic, i.e. it can be explained by a single, major gene variation, while more often variations in multiple minor genes contribute to the development of ATS. Mutations in the single gene coding for LDL receptor cause the appearance of Familial Hypercholesterolaemia [1]. Similarly, a mutation in the lipoprotein lipase gene results in elevated levels of triglycerides and decreased levels of HDL cholesterol, possibly leading to combined hyperlipidemia [2–4]. Moreover, several additional genetic variations have recently been identified, including the apolipoprotein E gene [5], the apolipoprotein (a) gene [6], the DD genotype of the angiotensin converting enzyme (ACE) [7], as well as genes regulating homocysteine metabolism and coding for methionine and homocysteine converting enzymes [8, 9]. The genetic determinants of ATS have been reviewed by Lusis [10], Doevendans et al. [11] and, more recently, by ourselves [12].

PPARs and LXRs: their role in atherogenesis

During the last few years, the knowledge concerning the role played by PPARs and LXRs in the development of ATS has rapidly grown [13–18]. The nuclear receptors PPAR alpha, gamma and beta/delta have been identified as transcription factors exerting modulatory actions in vascular cells. They belong to the nuclear receptor family of lingand-activated transcription factors.

PPAR alpha is activated by polyunsaturated fatty acids and oxidized derivatives, and by drugs of the fibrate family. PPAR alpha regulates the expression of genes involved in fatty acid beta-oxidation and energy homeostasis. Recently, it has been shown that PPAR alpha activation promotes cholesterol efflux from macrophages by induction of the ATP-binding cassette transporter A1 (ABCA1) pathway. Further, it has been demonstrated that PPAR alpha agonists, such as fibrates, exert anti-inflammatory and anti-thrombotic actions in the vessel wall, decrease trigycerides and elevate HDL cholesterol [19, 20]. Thus, PPAR alpha agonists could decrease the progression of ATS. This has been confirmed by clinical studies which showed that fibrates were able to reduce atherosclerotic plaque formation and the event rate of coronary artery disease [15, 19–21].

PPAR gamma is a key regulator of glucose homeostasis and adipogenesis. PPAR gamma ligands include naturally occurring fatty acids derivatives and insulin-sensitizing drugs such as glitazones. Recent data rewieved by Collins [22] have shown that PPAR gamma ligands have numerous pleiotropic vascular effects. They improve endothelial cell function, regulate proliferation and migration of vascular smooth muscle cells, and inhibit vascular inflammation. Furthermore, in macrophages they downregulate the production of inflammatory cytokines and the expression of SR-A and CD36 receptors. In animal models, PPAR gamma ligands have also been shown to minimize the development of ATS [22].

At present, no data are available on the possible effects on atherogenesis of *PPAR beta/delta* agonists, such as polyunsaturated fatty acids, prostaglandins and synthetic compounds. Their anti-inflammatory effects do not inhibit the development of ATS in hypercholesterolemic LDLR-deficient mice, and the activation of PPAR beta/delta can even be pro-atherogenic [23].

LXR alpha/beta are nuclear sterol sensors that play important roles in the regulation of cholesterol homeostasis in peripheral cells, including

macrophages. They positively regulate the expression of genes controlling cholesterol absorption, excretion, catabolism and cellular efflux in target organs, including the liver, the small intestine and macrophages [14, 24, 25]. In particular, LXRs regulate the expression of ABCA1, lipoprotein lipase (LPL), cholesteryl ester transfer protein (CEPT) and phospholipid transfer protein (PLTP). However, LXR agonists in mice stimulate fatty acid biosynthesis and cause an increase in circulating triglyceride levels, at least in part as a consequence of inducing the expression and activity of sterol response element binding protein 1c (SREBP-1c) [26, 27]. On the other hand, recent studies have demonstrated [28, 29] that LXR agonists can reduce the development of ATS in mice, raising the possibility that these compounds might be useful in humans if the effects on circulating triglyceride levels could be reduced through development of selective modulators [30].

In conclusion, emerging evidence supports the concept that both PPARs and LXRs may modulate the development and clinical course of ATS.

Oxidant stress and endothelial cell dysfunction

There is a wide consensus that ATS is associated with lipid and protein oxidation in the vascular wall [31–34]. In particular, according to the oxidative modification hypothesis of ATS, LDL oxidation is an early event that significantly contributes to atherogenesis. More recently, the hypothesis has evolved to focus on the oxidation of specific proinflammatory LDL phospholipids containing arachidonic acid. These are recognized by the innate immune system both in animals and humans [32]. The levels of specific oxidized lipids and lipoproteins, as well as the levels of antibodies to these lipids, may be useful markers of the susceptibility to atherogenesis.

At present, there is little information about the potential role of HDL oxidation in atherogenesis. Bergt et al. [34] have suggested that myeloperoxidase secreted by phagocytes promotes HDL oxidation in the human artery wall, which may favor atherogenesis by counteracting the established anti-atherogenetic effects of HDL. In particular, oxHDL and oxidized lipid-free apolipoprotein A-I, the major protein of HDL, are less able to remove cholesterol from cultured cells by the pathway requiring the cell membrane transporter ATP-binding cassette transporter A-I.

At sites of inflammation, the local cellular environment is enriched with cytokines, chemoattractant chemokines and reactive oxygen species (ROS). A major source of ROS is represented by the activated leukocytes that are found adherent to the endothelium. The acute and chronic oxidant stress associated with ROS leads to endothelial activation/dysfunction and plays a critical role in the pathophysiology of several diseases involving arterial walls, including diabetes, hypertension and ATS itself. Many excellent reviews regard ROS as signaling molecules, as well as the oxidative modification hypothesis of atherogenesis [35, 36].

The most important role among ROS is probably played by superoxide. Potential sources of vascular superoxide include NAD(P)H-dependent oxidases, xantine oxidase, lipoxygenase, mitochondrial oxidases and uncoupled nitric oxide (NO)-synthases [33]. Traditional risk factors, angiotensin II, inflammatory cytokines and low shear stress are all powerful activators of these enzymes and, consequently, oxidant stress inducers. NAD(P)H oxidases are present in vascular smooth muscle cells (VSMCs), endothelial cells and neutrophils, and represent the principal source of superoxide production in some animal models of ATS [37] and hypertension [38]. Increased superoxide production by the NAD(P)H oxidase system contributes to reduced NO bioactivity and endothelial dysfunction both in experimental models and in patients with systemic risk factors for ATS. In addition to oxidative inactivation of NO by ROS [36], there are several other potential causes of impaired vascular NO release and/or bioactivity. These include modifications of the substrate L-arginine for the constitutive endothelial cell-L arginine NO synthase (eNOS) system, and alterations of eNOS expression and NO signaling.

Is mitochondrial dysfunction an early event in atherogenesis?

Excess Reactive oxygen and nitrogen Species (RS) generation has been associated with vascular lesion formation and functional defects [39–41]. There is evidence that common risk factors for coronary artery disease (CAD) are associated with increased levels of RS [42–47]. Recent studies have

focused the role that mitochondria could play in the vascular changes occurring in atherogenesis. In fact, mitochondria are both important sources and targets for RS [45, 46]. Moreover, in addition to playing a role in metabolism, they also contribute to signal transduction pathways, and their activities in cells with relatively low energy demands, such as the endothelium, have received growing attention [48, 49]. The mitochondrial electron transport chain is both the source of the ATP generated via oxidative phosphorylation, and the main source of cellular ROS, such as superoxide and hydrogen peroxide (a side reaction in the electron transport chain) [50–52]. It has been shown that RS can cause mitochondrial injury *in vitro*, ranging from mitochondrial DNA damage and decreased adenine nucleotide translocase (ANT) activity, to alterations of mitochondrial proteins such as manganese superoxide dismutase [53–56]. Mitochondrial DNA encodes genes for oxidative phosphorylation, and ANT is a key factor for the translocation of adenine nucleotides across the inner mitochondrial membrane. Thus, both are essential for ATP production.

Mitochondrial damage contributes to further formation of ROS, which can compromise several metabolic processes that influence both endothelial and vascular smooth muscle cell function, all key components of atherogenesis [56].

Finally, recent data [47, 56] have suggested that oxidative damage to the mitochondrial genome may be an early event in the initiation of atherosclerotic lesions.

Effects of lipids and oxidized LDL on intimal cells

Elevated total and LDL cholesterol levels can impair endothelium-dependent vasodilation in peripheral vessels, resistance vessels and coronary arteries, even in the absence of ATS plaques. On the other hand, lowering LDL cholesterol can normalize impaired vasodilator responses [57]. An altered response to acetylcholine has been demonstrated in patients with high levels of plasma triglycerides, but normal LDL cholesterol levels [58]. This finding suggests that endothelial function may also be affected by lipid parameters other than LDL-cholesterol. Reduced NO bioavailability contributes to these effects, since a normalization of the vasodilator response can also be achieved by supplementation with the eNOS substrate L-arginine [57].

Many of the deleterious effects of LDL cholesterol are mediated by the oxidation of LDL particles. Oxidized LDL may favor leukocyte adhesion to endothelial cells through the stimulation of a number of cytokines, such as TNF alpha and interleukin-1 (IL-1) which, in turn, induce the surface expression of adhesion molecules [59–67]. This cytokine induced expression of adhesion molecules is inhibited by HDL and apo A1 [68]. Oxidized LDL can enhance the release from the endothelium of endothelin-1 (ET1) [69–71], a potent vasoconstrictor peptide that also acts as a mitogen on SMCs. Oxidized LDL also stimulates SMC proliferation by inducing expression of basic fibroblast growth factor in endothelial and smooth muscle cells [72]. Moreover, oxidized LDL induces SMC migration by increasing the expression of platelet-derived growth factor (PDGF) in endothelial cells, SMCs and macrophages [73–76]. Oxidized LDL also stimulates platelet adhesion and aggregation by decreasing prostacyclin (PGI2) production [77, 78]. Moreover, oxidized LDL enhances the procoagulant activity of the endothelium by inducing the release of tissue factor, reducing thrombomodulin transcription and suppressing protein C activation [79–81]. Oxidized LDL also stimulates apoptosis in endothelial, smooth muscle and foam cells, and thereby contributes to plaque rupture [82–84]. Finally, oxidized or modified LDL can be taken up by scavenger surface receptors of macrophages, leading to massive cholesterol accumulation.

Recruitment of mononuclear leucocytes

The recruitment of mononuclear leucocytes is one of the earliest events in the formation of atherosclerotic lesions. Several groups of adhesion molecules expressed on the surface of endothelial cells, such as the selectins [85–90] and the vascular cell adhesion molecules (VCAM) of the immunoglobulin superfamily [91–95], mediate the sticking of leucocytes to the endothelium.

Cook-Mills et al. [96] have suggested that VCAM-1 activates endothelial cell NAD(P)H oxidase, which is required for lymphocyte migration, through the mobilization of intracellular and extracellular calcium and the activation of the

Rac1 GTPase. Eriksson [97] has recently focused the mechanisms of leukocyte recruitment in atherosclerotic lesions. The focal increase in adhesion molecule and cytokine expression is mainly regulated by some constituents of oxidatively modified LDL (oxLDL), such as oxidized phospholipids [98, 99]. Growing evidence also indicates that the adhesive function of platelets represents an important recruitment mechanism in ATS, since targeted deficiency of P-selectin in platelets reduces ATS in mice. Moreover, platelets increase monocyte recruitment by secreting chemokines such as platelet factor 4 or RIANTES (regulated on activation, normal T-cell expressed and secreted), which trigger monocyte arrest in atherosclerotic lesions. A similar effect was also demonstrated for the fractalkine receptor CX3CRI and the chemioattractant LTB4. Endothelial nitric oxide (NO) can reduce leukocyte adhesion and migration into the intima at a transcriptional level, therefore acting as an anti-inflammatory mediator. The local formation of NO should also limit the ability of atherogenetic stimuli to augment the expression of adhesion molecules, such as VCAM-1 and macrophage chemoattractant protein-1 (MCP-1), though the activation of the nuclear factor-kB (NF-kB) system [100]. Thus, several adhesive and signalling mechanisms can be involved in leukocyte recruitment in ATS.

Once adherent, leucocytes migrate into the intima by the action of chemoattractant chemokines [101, 102]. The macrophages recruited in the arterial wall take up modified LDL and become lipid-laden cholesterol-engorged foam cells. The continued inflammation can lead to cellular necrosis within the lesion, and further recruitment of inflammatory cells and release of cytokines, proteolytic enzymes and growth factors. Although these factors may contribute to the development of ATS, a major part of its etiology still needs to be established.

Rho and Ras in ATS

Some heterotrimeric G proteins, such as the Rho GTPases (including Rho A, Rho B, Rac and Cdc42) and the Ras superfamily of GTPases, function as critical relays in the intracellular transduction of many important signals emanating from membrane receptors. Small GTPases are involved in the regulation of various cellular processes, such as cell motility [103], apoptosis and proliferation [104–106]. Moreover, Rho and Rho-Kinase can regulate endothelial tissue factor induction by thrombin, which plays a pivotal role in thrombus formation in acute coronary syndromes [107]. In addition, Rho proteins negatively regulate endothelial NO synthase (eNOS) [108] and stimulate vascular tone by increasing smooth muscle Ca (2+) sensitivity. Finally, Rho proteins are also modulators of the scavenger receptor CD36, which plays important roles in atherosclerosis, inflammation, thrombosis and angiogenesis [109]. All the above processes may be enhanced by oxidized LDL, which has been suggested to be an activator of the Rho A pathway.

A post-translational modification of these proteins occurs in the presence of isoprenoids, such as farnesylpyrophosphate (FPP), a branch point in the cholesterol metabolic pathway, and geranylgeranylpyrophosphate (GGPP), a derivative of FPP [110]. Through isoprenylation small GTPases are converted from an inactive (cytosolic) state to a membrane-bound (active) state [108, 111, 112]. Interestingly, the inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase – i.e. the statins – interfere with the biosynthesis of FPP, which is involved in the post-translational lipidation of Ras GTPases, and of GGPP, which is required for the posttranslational lipidation of Rho proteins [113, 114]. Thus, statins upregulate eNOS through the inhibition of Rho protein geranylgeranylation [111]. Moreover, by preventing the geranyl geranyl-dependent translocation of Rac 1 from the cytosol to cell membrane [106, 115], statins may interfere with the activation of NAD(P)H-oxidase, a prominent mechanism of ROS production [116, 117]. Finally, statins downregulate plasminogen activator inhibitor-1 (PAI-1) synthesis in cultured human monocytes via blockade of Rho/Rho Kinase signalling [118]. Overall, these findings provide new insights into the multiple pleiotropic anti-atherogenic actions of statins.

Other G proteins in atherogenesis

Endothelial NOS is activated by a number of stimuli, such as bradykinin, acetylcholine, thrombin, histamine, substance P, ATP, vascular endothelial growth factor and fluid shear stress [119].

The hypothesis has been advanced that eNOS is activated in endothelial cells as a consequence of reversible inhibitory interactions of the above stimuli with G-protein-coupled receptors. On the contrary, the Gi protein may activate eNOS, and its expression in human coronary arteries may be impaired by hypertension, hypercholesterolaemia and age [120]. Also, oxidized LDL could inhibit Giα 2 protein sub-unit expression in tissue cultures [119], probably through changes in membrane fluidity [36]. It has been suggested that the mitogenic effect of oxidized LDL in canine cultured smooth muscle cells (SMC) is mediated by a G-protein-coupled receptor [121–123]. Similarly, Zhao et al. [123] demonstrated that β-migrating VLDL activates SMC proliferation via the G protein-coupled transactivation of the tyronine kinase receptor.

Caveolin and cholesterol trafficking

Caveolae are vescicular organelles (50–100 nm in diameter) which are particularly abundant in cells of the cardiovascular system, such as endothelial cells, SMCs, macrophages, cardiac myocytes and fibroblasts. In these cells, caveolae are involved in protein trafficking, signal transduction and cholesterol homeostasis. Caveolins, the structural proteins of caveolae membrane domains, can interact with a number of downstream signaling molecules, holding them in an inactive conformation until activation by an appropriate stimulus occurs. In particular, caveolins can bind cholesterol, or proteins such as receptors, G-proteins, and NO synthases. Thus, caveolae and caveolins can play a significant role in several human pathobiological conditions, including ATS.

Caveolin is involved in cholesterol trafficking through palmitoylation [124] or interaction with proteins such as heat-shock protein 56 [125, 126]. Everson and Smart [125] focused on the interactions among Caveolin-1, eNOS, lipoprotein receptors, heterotrimeric G protein-coupled receptors, cholesterol and a calcium channel, which form activation complexes associated with cholesterol-rich caveolae. Oxidized LDL might disrupt this organization of caveolae by removing caveola cholesterol, while HDL prevents cholesterol depletion, probably serving as a sterol donor to replace the cholesterol content transferred to oxidized LDL [124]. Cholesterol depletion in cell surface caveolae can disrupt the caveolin activation complex. This disruption could be associated with eNOS internalization and decrease in NO production, suggesting a possible mechanistic basis for some of the molecular changes observed in vascular disease [125].

HDL and ATS

HDL cholesterol is inversely and independently associated with the risk of coronary artery disease. HDL may exert several potentially anti-atherogenic effects [127, 128], and low HDL cholesterol concentrations may lead to endothelial dysfunction and increased LDL oxidation [129].

Krause and Auerbach [130] and von Eckardstein et al. [131] have reviewed the role of HDL in reverse cholesterol transport from peripheral cells (such as macrophage foam cells) to the liver and steroidogenic organs, for the synthesis of lipoproteins, bile acids, vitamin D and steroid hormones [127, 128]. This process involves many key steps and lipoprotein interconversions. The role of macrophages is also important, since they actively secrete apo E which may promote cholesterol efflux to HDL [132]. SR-B1, a member of the class B scavenger receptor family, has been identified as an HDL receptor capable to mediate the selective uptake of cholesteryl esters from HDL by the liver both *in vitro* and *in vivo* [133]. The ABCA1 protein is known to belong to a superfamily of membrane transporters that bind and hydrolyse ATP to drive diverse substrates across membranes. Cholesterol and phospholipids are ABCA1 substrates, and their efflux may be facilitated by the binding of apo A1 to ABCA1. The initial step in reverse cholesterol transport is the release of superfluous free cholesterol and phospholipids from the cell plasma membrane to both apolipoproteins and HDL. This pathway is crucial to counteract cholesterol deposition in monocyte-macrophages in the arterial wall, and ineffective cholesterol efflux causes the formation of foam cells [134]. Wang et al. [135] also suggested that cholesterol efflux can be dissociated from phospholipid efflux. Frank et al. [136] showed that caveolin 1, a structural component of caveola membranes, can negatively influence the HDL-mediated cholesterol efflux, suggesting its proatherogenic role regarding the uptake and/or

transcytosis of modified lipoproteins. Oliver et al. [137] suggested a novel role for peroxisome proliferator-activated receptor (PPAR) delta agonists in increasing the expression of the reverse cholesterol transporter ABCA1 and inducing apo A1-specific cholesterol efflux. Interferon-gamma can induce the accumulation of cholesterol esters in macrophages through the inhibition of ABCA1 [138]. The effluxed cholesterol is esterified by the enzyme lecithin:cholesterol acyltransferase (LCAT) to form cholesteryl esters. Then, cholesteryl esters are transferred to apo B-containing lipoproteins by the cholesteryl ester transfer protein (CETP). The phospholipid transfer protein (PLTP) transfers phospholipids from triglyceride-rich lipoproteins to HDL during lipolysis [139]. The initial products are small HDL particles, which grow into larger HDL2 particles by the action of LCAT and PLTP.

Lipids are removed from the circulation by the scavenger receptor B1 (SR-B1), or through apoprotein uptake by apo E or apo A-1 receptors. Other indirect pathways involve the actions of CETP, hepatic lipase and endothelial lipase [140–146]. Small apolipoproteins are thus generated that can move from plasma into the extracellular space [147–149] and serve as acceptors of cellular lipids, thus regenerating HDL. These small particles can also be removed from plasma by the kidneys [150–152].

Interestingly, the determinants of HDL metabolism and reverse cholesterol transport, such as LCAT and paraoxonase (PON), are inactivated by mildly or extensively oxidized LDL, resulting in an impairment of cholesterol efflux [153]. Aviram and Rosenblat [154] have suggested that PON1 and PON3, which are both associated with HDL, protect serum lipids from oxidation, presumably as a result of their ability to hydrolyze specific oxidized lipids. In particular, PON1 has been shown to inhibit cholesterol influx in mice macrophages by reducing the formation and uptake of oxLDL, and increasing the breakdown of oxidized lipids. Furthermore, PON1 inhibits cholesterol synthesis and stimulates HDL-mediated cholesterol efflux from macrophages, whereas PON2 and PON3 protect against oxidative stress. Under oxidative stress, serum PON1 and PON3 are inactivated, but PON2 expression and activity are increased, a probable compensatory mechanism. Interventions aimed at increasing cellular and humoral paraoxonases by pharmacological or dietary means can promote the reduction of foam cell formation and attenuate ATS development [154], suggesting that paraoxonase activity could play an important role in atherogenesis.

The mature ATS lesion and atherothrombosis

The mature atherosclerotic plaque is generated during the silent phase of ATS. The classical plaque involves a central core of foam cells and lipids that are both intra and extracellular. The extracellular cholesterol is arranged in the so-called cholesterol clefts. Plaques contain also T-limphocytes, a considerable amount of necrotic debris and, overlying the central core, variable proportions of connective extracellular tissue matrix produced by SMCs. Solid stable plaques, almost entirely composed of connective tissue, result in a progressive narrowing of the arterial lumen and decrease in blood flow. Unstable lipid-rich plaques have a thin fibrous cap and a large core of extracellular lipids, that may occupy up to 50–60% of the entire plaque volume. The core is surrounded by inflammatory cells such as lymphocytes, mast cells and macrophages expressing inflammatory cytokines, the procoagulant tissue factor and plasmin-activated metallo proteinases. The atherosclerotic plaque biology is mainly modulated by an immune-mediated and lipid-related chronic inflammatory process. Plaque vulnerability strongly relates to intraplaque inflammation [155]. In particular, plaques prone to rupture or surface erosions contain more inflammatory cells than stable plaques. This has been confirmed in atherotomy specimens from patients with coronary artery diseases, in which a direct relationship between the number of inflammatory cells and the severity of the coronary syndrome was demonstrated [156]. Also, the number of T-lymphocytes expressing IL-2 receptor activity, a marker of recent-onset activation, was significantly related to the severity of the syndrome [156]. Libby and his group [157–161] showed that the functional state of atheroma is the major determinant of the propensity of a plaque to rupture and to cause acute coronary syndromes. T-lymphocytes within the plaque may modulate the inflammatory response by sending signals through the mediators of inflammation, i.e. cytokines, that convey messages to both SMCs and macrophages. The SMCs

respond to T-lymphocyte signals by decreasing the synthesis of new collagen fibrils. Macrophages respond to these signals by increasing interstitial collagenases, known as matrix metalloproteinases (MMP), which initiate collagen breakdown. The loss of interstitial collagen (up to 60% of the total protein of plaque) by degradation of subendothelial extracellular matrix, renders the atheroma prone to rupture. Dysregulated MMP activity can also be due to imbalance of several endogenous inhibitors [159]. Moreover, recent studies have demonstrated the overexpression of members of the MMP subfamily, such as MMP-1, MMP-8, MMP-2 and MMP-13, in various vascular cell types, including endothelial cells, SMCs and mononuclear monocytes [159].

The rupture of the atherosclerotic plaque exposes the lipid core to the blood, triggering for thrombus formation. The thrombogenicity of the core is likely due to the presence of tissue factor (TF) [160]. TF binds to factors VII and X, resulting in accelerated conversion of factors IX and X to active factors. Furthermore, TF promotes the generation of thrombin, which converts fibrinogen to fibrin. Schonbeck et al. [161] demonstrated that CD40, a member of the TNF receptor family, is able to induce TF expression in human vascular SMCs after ligation with the native CD40 ligand (CD40L) derived from activated T-lymphocytes. It has recently been shown that CD40L is expressed in endothelial cells and macrophages, also inducing TF in these cells [161]. The expression of TF by several cell types suggests that thrombosis may develop in the presence of inflammation and superficial erosion of atheroma, even without the direct contact of blood with the central core of the plaque, as is the case when the fibrous cap ruptures [161]. Marx et al. [162] recently demonstrated that PPAR alpha activators can inhibit TF expression and activity in human monocytes, and thus may potentially reduce the thrombogenicity of atherosclerotic lesions. These data provide new insight into how PPAR alpha activators, such as fibric acid derivatives and certain fatty acids, might modulate atherothrombosis in patients with vascular disease.

Therapeutic implications

As a consequence of the "oxidative modification hypothesis" of ATS, several studies on possible anti-oxidant treatment strategies have been conducted. However, such strategies did not appear to significantly ameliorate the rate of coronary events. In the HOPE trial, no significant effect of anti-oxidant vitamins was demonstrated on cardiovascular disease [163], and the same result was obtained in the Heart Protection Study (HPS) [164]. Thus, the question arises whether oxidation of LDL lipids and/or proteins is really relevant to human ATS development. Ricciarelli et al. [165] suggested that vitamin E treatment should be started much earlier in selected people, continue for a long period at high doses, and be associated with vitamin C (that could counteract the pro-oxidant effects of α-tocopherol). Heinecke [166] reported that most animal studies have focused on the effects of anti-oxidants on the formation of fatty streaks, whereas the effects on the progression of ATS have been much less investigated. Moreover, little is known about the influence of oxidant stress on clinical events in humans, such as the rupture of atherosclerotic plaques [167, 168]. Finally, some observations in animals suggest that ATS development and lipoprotein oxidation might be dissociated [169]. Stocker and Keney [170] suggest that "it remains to be established that oxidative events are a cause rather than an injurious response to atherogenesis. In this context, inflammation needs to be considered as a primary process of ATS, and oxidative stress as a secondary event". To address this issue, these authors have proposed an "oxidative response to inflammation" model to reconcile the response-to-injury and oxidative modification hypotheses of ATS.

Several studies (reviewed by Vaughan et al. [171], and others [172, 173]) have shown that the most potent lipid-lowering drugs, the HMG-CoA reductase inhibitors (statins), can ameliorate coronary artery disease morbidity and mortality in the setting of both hypercholesterolaemia and normocholesterolaemia. In particular, recent studies have shown that these drugs can decrease LDL oxidation. However, both *in vitro* and animal models have shown that the inhibition of Rho GTPases with statins could induce a decrease in endothelial NO production [174], which is in contrast with the results of other studies [111]. Thus, further evidence is needed before a full assessment of the clinical importance of isoprenylation blockage with therapeutic concentrations of statins in humans can be made. Further, statins

can limit endothelial proinflammatory factors, suggesting new possible mechanisms in the prevention of early lesions, vascular restenosis and plaque dysruption [175–177].

From this observation, another question arises: is it possible to use classical anti-inflammatory agents for the prevention of the inflammatory process underlying ATS? Several experimental studies have been conducted on anti-inflammatory drugs such as aspirin, ibuprofen, acetaminophen and desamethasone, showing beneficial effects on proinflammatory components in the early development of atherosclerotic lesions. In particular, when continously administrated, aspirin has been shown to attenuate ATS development in apolipoprotein E-deficient mice [178]. In humans, however, the beneficial effects of aspirin in reducing ischemic events have mainly been attributed to its anti-platelet action [179].

A family history of coronary artery disease (CAD) is a well known risk factor for the development of the disease, which may act even in the absence of other traditional risk factors. Could the knowledge of specific genetic components be of relevance in the management of ATS? Two genetic mutations/variations favouring an accelerated progression of atherosclerosis concern the genes of lipoprotein lipase and cholesteryl ester transfer protein and, in both cases, the deleterious effects of the mutation could be reversed by statin treatment [180]. This example suggests that in the near future the assessment of genetic factors will help to identify patients at high risk of progression of CAD, thereby allowing an optimal and opportune therapeutic strategy.

References

1. Goldstein J.L. and Brown M.S., LDL receptor locus and the genetics of familial hypercholesterolemia. Ann. Rev. Genet. 13: 259–289, 1979.
2. Jukema J.W., van Boven A.J., Groenemeijer B., Zwinderman A.H., Reiber J.H., Bruschke A.V., Henneman J.A., Molhoek G.P., Bruin T., Jansen H., Gagne E., Hayden M.R. and Kastelein J.J., The Asp9 Asn mutation in the lipoprotein lipase gene is associated with increased progression of coronary atherosclerosis. REGRESS Study Group, Interuniversity Cardiology Institute, Utrecht, The Netherlands. Regression Growth Evaluation Statin Study. Circulation 94: 193–1918, 1996.
3. Mailly F., Tugrul Y., Reymer P.W., Bruin T., Seed M., Groenemeyer B.F., Asplund-Carlson A., Vallance D., Winder A.F. and Miller G.J., A common variant in the gene for lipoprotein lipase (Asp9 → Asn). Functional implications and prevalence in normal and hyperlipidemic subjects. Arterioscler. Thromb. Vasc. Biol. 15: 468–478, 1995.
4. Pimstone S.N., Gagne S.E., Gagne C., Lupien P.J., Gaudet D., Williams R.R., Kotze M., Reymer P.W., Defesche J.C. and Kastelein J.J., Mutations in the gene for lipoprotein lipase. A cause for low HDL cholesterol levels in individuals heterozygous for familial hypercholesterolemia. Arterioscler. Thromb. Vasc. Biol. 15: 1704–1712, 1995.
5. Dzau V.J., Gibbons G.H., Cooke J.P. and Omoigui N., Vascular biology and medicine in the 1990s. scope, concepts, potentials and perspectives. Circulation 87: 705–719, 1993.
6. van den Ende A., van der Hoek Y.Y., Kastelein J.J., Koschinsky M.L., Labeur C. and Rosseneu M., Lipoprotein [a]. Adv. Clin. Chem. 32: 73–134, 1996.
7. Ribichini F., Steffenino G., Dellavalle A., Matullo G., Colajanni E., Camilla T., Vado A., Benetton G., Uslenghi E. and Piazza A., Plasma activity and insertion/deletion polymorphism of angiotensin I-converting enzyme. A major risk factor and a marker of risk for coronary stent restenosis. Circulation 97: 147–154, 1998.
8. Fross P., Blom H.J., Milos R., Goyette P., Sheppard C.A., Matthews R.G., Boers G.J., den Heijer M., Kluijtmans L.A. and van den Heuvel L.P., A candidate genetic risk factor for vascular disease. A common mutation in methylenetetrahydrofolate reductase. Nat. Genet. 10: 111–113, 1995.
9. Kluijtmans L.A., Kastelein J.J., Lindemans J., Boers G.H., Heil S.G., Bruschke A.V., Jukema J.W., van den Heuvel L.P., Trijbels F.J., Boerma G.J., Verheugt F.W., Willelms F. and Blom H.J., Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. Circulation 96: 2573–2577, 1997.
10. Lusis A.J., Atherosclerosis. Nature 407: 233–241, 2000.
11. Doevendans P.A., Jukema W., Spiering W., Defesche J.C. and Kastelein J.J.P., Molecular genetics and gene expression in atherosclerosis. Int. J. Cardiol. 80: 161–172, 2001.
12. Puddu P., Cravero E., Puddu G.M. and Muscari A., Genes and atherosclerosis: at the origin of the predisposition. Int. J. Clin. Pract. 59: 462–472, 2005.
13. Puddu P., Puddu G.M. and Muscari A., Peroxisome proliferator–activated receptors: are they involved in atherosclerosis progression? Int. J. Cardiol. 90: 133–140, 2003.
14. Li A.C. and Glass C.K., PPAR- and LXR-dependent pathways controlling lipid metabolism and the development of atherosclerosis. J. Lipid Res. 45: 2161–2173, 2004.
15. Marx N., Duez H., Fruchart J.C. and Staels B., Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. Circ. Res. 14(94) 1168–1178, 2004.
16. Neve B.P., Fruchart J.C. and Staels B., Role of peroxisome proliferator-activated receptors (PPAR) in atherosclerosis. Biochem. Pharmacol. 60: 1245–1250, 2000.
17. Duez H., Fruchart J.C. and Staels B., PPAR in inflammation, atherosclerosis and thrombosis. J. Cardiovasc. Risk 8: 187–194, 2001.
18. Elangbam C.S., Tyler R.D. and Lightfoot R.M., Peroxisome proliferator-activated receptors in atherosclerosis and inflammation-an update. Toxicol. Pathol. 29: 224–231, 2001.

19. van Raalte D.H., Li M., Pritchard P.H. and Wasan K.M., Peroxisome proliferator-activated receptor (PPAR)-alpha: a pharmacological target with a promising future. Pharm. Res. 21: 1531–1538, 2004.
20. Chinetti G., Lestavel S., Fruchart J.C., Clavey V. and Staels B., Peroxisome proliferator-activated receptor alpha reduces cholesterol esterification in macrophages. Circ. Res. 92: 212–217, 2003.
21. Israelian-Konaraki Z. and Reaven P.D., Peroxisome proliferator-activated receptor-alpha and atherosclerosis: from basic mechanisms to clinical implications. Cardiology 103: 1–9, 2005.
22. Collins A.R., Pleiotropic vascular effects of PPARgamma ligands. Drug News Perspect. 16: 197–204, 2003.
23. Lee D.L., Webb R.C. and Jin L., Hypertension and RhoA/Rho-kinase signaling in the vasculature: highlights from the recent literature. Hypertension 44: 796–799, 2004.
24. Jaye M., LXR agonists for the treatment of atherosclerosis. Curr. Opin. Investig. Drugs 4: 1053–1058, 2003.
25. Repa J.J., Berge K.E., Pomajzl C., Richardson J.A., Hobbs H. and Mangelsdorf D.J., Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta. J. Biol. Chem. 277: 18793–18800, 2002.
26. Repa J.J., Liang G., Ou J., Bashmakov Y., Lobaccaro J.M., Shimomura I., Shan B., Brown M.S., Goldstein J.L. and Mangelsdorf D.J., Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. Genes Dev. 14: 2819–2830, 2000.
27. Mak P.A., Laffitte B.A., Desrumaux C., Joseph S.B., Curtiss L.K., Mangelsdorf D.J., Tontonoz P. and Edwards P.A., Regulated expression of the apolipoprotein E/C-I/C-IV/C-II gene cluster in murine and human macrophages. A critical role for nuclear liver X receptors alpha and beta. J. Biol. Chem. 277: 31900–31908, 2002.
28. Joseph S.B., McKilligin E., Pei L., Watson M.A., Collins A.R., Laffitte B.A., Chen M., Noh G., Goodman J., Hagger G.N, Tran J., Tippin T.K., Wang X., Lusis A.J., Hsueh W.A., Law R.E., Collins J.L., Willson T.M. and Tontonoz P., Synthetic LXR ligand inhibits the development of atherosclerosis in mice. Proc. Natl. Acad. Sci. USA 99: 7604–7609, 2002.
29. Tangirala R.K., Bischoff E.D., Joseph S.B., Wagner B.L., Walczak R., Laffitte B.A., Daige C.L., Thomas D., Heyman R.A., Mangelsdorf D.J., Wang X., Lusis A.J., Tontonoz P. and Schulman I.G., Identification of macrophage liver X receptors as inhibitors of atherosclerosis. Proc. Natl. Acad. Sci. USA 99: 11896–11901, 2002.
30. Schultz J.R., Tu H., Luk A., Repa J.J., Medina J.C., Li L., Schwendner S., Wang S., Thoolen M., Mangelsdorf D.J., Lustig K.D. and Shan B., Role of LXRs in control of lipogenesis. Genes Dev. 14: 2831–2838, 2000.
31. Stocker R. and Keaney J.F. Jr, Role of oxidative modifications in atherosclerosis. Physiol. Rev. 84: 1381–478, 2004.
32. Navab M., Ananthramaiah G.M., Reddy S.T., Van Lenten B.J., Ansell B.J., Fonarow G.C., Vahabzadeh K., Hama S., Hough G., Kamranpour N., Berliner J.A., Lusis A.J. and Fogelman A.M., The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. J. Lipid. Res. 2004(45) 993–1007, 2004.
33. Madamanchi N.R., Vendrov A. and Runge M.S., Oxidative stress and vascular disease. Arterioscler. Thromb. Vasc. Biol. 25: 29–38, 2005.
34. Bergt C., Pennathur S., Fu X., Byun J., O'Brien K., McDonald T.O., Singh P., Anantharamaiah G.M., Chait A., Brunzell J., Geary R.L., Oram J.F. and Heinecke J.W., The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. Proc. Natl. Acad. Sci. USA 31(101) 13032–13037, 2004.
35. Chisolm G.M. and Steinberg D., The oxidative modification hypothesis of atherogenesis. An overview. Free Radic. Biol. Med. 28: 1815–1826, 2000.
36. Harrison D.G., Cellular and molecular mechanisms of endothelial cell dysfunction. J. Clin. Invest. 100: 2153–2157, 1997.
37. Guzik T.J., West N.E.J., Black E., McDonald D., Ratnatunga C., Pillai R. and Channon K.M., Vascular superoxide production by NAD(P)H oxidase. Associaton with endothelial dysfunction and clinical risk factors. Circ. Res. 86: e85–e90, 2000.
38. Rajagopalan S., Kurz S., Munzel T., Tarpey M., Freeman B.A., Griendling K.K. and Harrison D.G., Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. J. Clin. Invest. 97: 1916–1923, 1996.
39. Ross R., The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362: 801–809, 1993.
40. Berliner J.A. and Heinecke J.W., The role of oxidized lipoproteins in atherogenesis. Free Radic. Biol. Med. 20: 707–727, 1996.
41. Freeman B.A., White C.R., Gutierrez H., Paler-Martinez A., Tarpey M.M. and Rubbo H., Oxygen radical-nitric oxide reactions in vascular diseases. Adv. Pharmacol. 34: 45–69, 1995.
42. Alexander R.W., Atherosclerosis as disease of redox-sensitive genes. Trans. Am. Clin. Climatol. Assoc. 109: 129–145, 1998.
43. Ito H., Torii M. and Suzuki T., Decreased superoxide dismutase activity and increased superoxide anion production in cardiac hypertrophy of spontaneously hypertensive rats. Clin. Exp. Hypertens. 17: 803–816, 1995.
44. van Jaarsveld H., Kuyl J.M. and Alberts D.W., Exposure of rats to low concentration of cigarette smoke increases myocardial sensitivity to ischaemia/reperfusion. Basic Res. Cardiol. 87: 393–399, 1992.
45. Esposito L.A., Melov S., Panov A., Cottrell B.A. and Wallace D.C., Mitochondrial disease in mouse results in increased oxidative stress. Proc. Natl. Acad. Sci. USA 96: 4820–4825, 1999.
46. Wallace D.C., Mitochondrial genetics: a paradigm for aging and degenerative diseases?. Science 256: 628–632, 1992.
47. Ballinger S.W., Patterson C., Knight-Lozano C.A., Burow D.L., Conklin C.A., Hu Z., Reuf J., Horaist C., Lebovitz R., Hunter G.C., McIntyre K. and Runge M.S., Mitochondrial integrity and function in atherogenesis. Circulation 106: 544–549, 2002.
48. Puddu P., Puddu G.M., Cravero E. and Muscari A., Mitochondrial dysfunction as an initiating event in atherogenesis – a plausible hypothesis. Cardiology 103: 137–141, 2005.
49. Ramachandran A., Levonen A.L., Brookes P.S., Ceaser E., Shiva S., Barone M.C. and Darley-Usmar V., Mito-

chondria, nitric oxide, and cardiovascular dysfunction. Free Radic. Biol. Med. 33: 1465–1474, 2002.
50. Chance B., Sies H. and Boveris A., Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 59: 527-605, 1979.
51. Papa S. and Skulachev V.P., Reactive oxygen species, mitochondria, apoptosis and aging. Mol. Cell. Biochem. 174: 305–319, 1997.
52. Shackelford R.E., Kaufmann W.K. and Paules R.S., Oxidative stress and cell cycle checkpoint function. Free Radic. Biol. Med. 28: 1387–1404, 2000.
53. Ballinger S.W., Patterson C., Yan C.N., Doan R., Burow D.L., Young C.G., Yakes F.M., Van Houten B., Ballinger C.A., Freeman B.A. and Runge M.S., Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. Circ. Res. 86: 960–966, 2000.
54. Yan L.J. and Sohal R.S., Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. Proc. Natl. Acad. Sci. USA 95: 12896–12901, 1998.
55. MacMillan-Crow L.A., Crow J.P. and Thompson J.A., Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. Biochemistry 37: 1613–1622, 1998.
56. Knight-Lozano C.A, Young C.G, Burow D.L, Hu Z.Y, Uyeminami D., Pinkerton K.E., Ischiropoulos H. and Ballinger S.W., Cigarette smoke exposure and hypercholesterolemia increase mitochondrial damage in cardiovascular tissues. Circulation 105: 849–854, 2002.
57. Dart A.M. and Chin-Dusting J.P.F., Lipids and the endothelium. Cardiovasc. Res. 43: 308–322, 1999.
58. Lewis T.V., Dart A.M. and Chin-Dusting J.P.F., Endothelium-dependent relaxation by acetylcholine is impaired in hypertriglyceridemic humans with normal levels of plasma LDL cholesterol. J. Am. Coll. Cardiol. 33: 805–812, 1999.
59. Parhami F., Fang Z.T., Fogelman A.M., Andalibi A., Territo M.C. and Berliner J.A., Minimally modified low density lipoprotein-induced inflammatory responses in endothelial cells are mediated by cyclic adenosine monophosphate. J. Clin. Invest. 92: 471–478, 1993.
60. Vora D.K., Fang Z.T., Liva S.M., Tyner T.R., Parhami F., Watson A.D., Drake T.A., Territo M.C. and Berliner J.A., Induction of P-selectin by oxidized lipoproteins. Separate effects on synthesis and surface expression. Circ. Res. 80: 810–818, 1997.
61. Smalley D.M., Lin J.H., Curtis M.L., Kobari Y., Stemerman M.B. and Pritchard K.A. Jr., Native LDL increase endothelial cell adhesiveness by inducing intercellular adhesion molecule-1. Arterioscler. Thromb. Vasc. Biol. 16: 585–590, 1996.
62. Lin J.H., Zhu Y., Liao H.L., Kobari Y., Groszek L. and Stemerman M.B., Induction of vascular cell adhesion molecule-1 by low-density lipoprotein. Atherosclerosis 127: 185–194, 1996.
63. Allen S., Khan S., Al-Mohanna F., Batten P. and Yacoub M., Native low-density lipoprotein-induced calcium transient trigger VCAM-1 and E-selectin expression in cultured human vascular endothelial cells. J. Clin. Invest. 101: 1064–1075, 1998.
64. Rajavashisth T.B., Andalibi A., Territo M.C., Berliner J.A., Navab M., Fogelman A.M. and Lusis A.J., Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. Nature 344: 254–247, 1990.
65. Johnson-Tidey R.R., McGregor J.L., Taylor P.R. and Poston R.N., Increase in the adhesion molecule P-selectin in endothelium overlying atherosclerotic plaques.Coexpression with intercellular adhesion molecule-1. Am. J. Pathol. 144: 952–961, 1994.
66. Khan B.V., Harrison D.G., Olbrych M.T., Alexander R.W. and Medford R.M., Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. Proc. Natl. Acad. Sci. USA 93: 9114–9119, 1996.
67. Sugiyama S., Kugiyama K., Ohgushi M., Fujimoto K. and Yasue H., Lysophosphatidylcholine in oxidized low-density lipoprotein increases endothelial susceptibility to polymorphonuclear leukocyte-induced endothelial dysfunction in porcine coronary arteries. Role of protein kinase C. Circ. Res. 74: 565–575, 1994.
68. Maier J.A., Barenghi L., Bradamante S. and Pagani F., Modulators of oxidized LDL-induced hyperadhesiveness in human endothelial cells. Biochem. Biophys. Res. Commun. 204: 673–677, 1994.
69. Myers D.E., Huang W.N. and Larkins R.G., Lipoprotein-induced prostacyclin production in endothelial cells and effects of lipoprotein modification. Am. J. Physiol. 271: C1504–C1511, 1996.
70. Haug C., Schmid-Kotsas A., Zorn U., Schuett S., Gross H.J., Gruenert A. and Bachem M.G., Endothelin-1 synthesis and endothelin b receptor expression in human coronary artery smooth muscle cells and monocyte-derived macrophages is up-regulated by low density lipoproteins. J. Mol. Cell Cardiol. 33: 1701–1712, 2001.
71. He Y., Kwan W.C. and Steinbrecher U.P., Effects of oxidized low density lipoprotein on endothelin secretion by cultured endothelial cells and pacrophages. Atherosclerosis 119: 107–118, 1996.
72. Lindner V., Lappi D.A., Baird A., Majack R.A. and Reidy M.A., Role of basic fibroblast growth factor in vascular lesion formation. Circ. Res. 68: 106–113, 1991.
73. Stiko-Rahm A., Hultgardh-Nilsson A., Regnstrom J., Hamsten A. and Nilsson J., Native and oxidized LDL enhances production of PDGF AA and the surface expression of PDGF receptors in cultured human smooth muscle cells. Arterioscler. Thromb. 12: 1099–1109, 1992.
74. Kohno M., Yokokawa K., Yasunari K., Minami M., Kano H., Hanehira T. and Yoshikawa J., Induction by lysophosphatidylcholine, a major phospholipid component of atherogenic lipoproteins, of human coronary artery smooth muscle cell migration. Circulation 98: 353–359, 1998.
75. Kim J.G., Taylor W.R. and Parthasarathy S., Demonstration of the presence of lipid peroxide-modified proteins in human atherosclerotic lesions using a novel lipid peroxidemodified anti-peptide antibody. Atherosclerosis 143: 335–340, 1999.
76. Mertens A. and Holvoet P., Oxidized LDL and HDL. Antagonists in atherothrombosis. FASEB J. 15: 2073–2084, 2001.
77. Thorin E., Hamilton C.A., Dominiczak M.H. and Reid J.L., Chronic exposure of cultured bovine endothelial cells to oxidized LDL abolishes prostacyclin release. Arterioscler. Thromb. 14: 453–459, 1994.
78. Li L.X., Chen J.X., Liao D.F. and Yu L., Probucol inhibits oxidized-low density lipoprotein-induced adhesion

of monocytes to endothelial cells by reducing P-selectin synthesis *in vitro*. Endothelium 6: 1–8, 1998.
79. Frei B. and Gaziano J.M., Content of antioxidants, preformed lipid hydroperoxides and cholesterol as predictors of the susceptibility of human LDL to metal ion-dependent and-independent oxidation. J. Lipid Res. 34: 2135–2145, 1993.
80. Penn M.S., Vui M.Z., Winokur A.L., Bethea J., Hamilton T.A., DiCorleto P.E. and Chisolm G.M., Smooth muscle cell surface tissue factor pathway activation by oxidized low-density lipoprotein requires cellular lipid peroxidation. Blood 96: 30556–33063, 2000.
81. Ishii H., Kizaki K., Horie S. and Kazama M., Oxidized low density lipoprotein reduces thrombomodulin transcription in cultured human endothelial cells through degradation of the lipoprotein in lysosomes. J. Biol. Chem. 271: 8458–8465, 1996.
82. Kockx M.M., Apoptosis in the atherosclerotic plaque. Quantitative and qualitative aspects. Arterioscler. Thromb. Vasc. Biol. 18: 1519–1522, 1998.
83. Heermeier K., Leicht W., Palmetshofer A., Ullrich M., Wanner C. and Galle J., Oxidized LDL suppresses NF-kappa B and overcomes protection from apoptosis in activated endothelial cells. J. Am. Soc. Nephrol. 12: 456–463, 2001.
84. Metzler B., Hu Y., Dietrich H. and Xu Q., Increased expression and activation of stress-activated protein kinases/c-Jun NH (2)-terminal protein kinases in atherosclerotic lesions coincide with p53. Am. J. Pathol. 156: 1875–1886, 2000.
85. Carlos T.M. and Harlan J.M., Leukocyte-endothelial adhesion molecules. Blood 84: 2068–2101, 1994.
86. Frenette P. and Wagner D., Adhesion molecules. New Engl. J. Med. 334: 1526–1529, 1996.
87. Vora D.K., Fang Z.T., Liva S.M., Tyner T.R., Parhami F., Watson A.D., Drake T.A., Territo M.C. and Berliner J.A., Induction of P-selectin by oxidized lipoproteins. Separate effects on synthesis and surface expression. Circ. Res. 80: 810–818, 1997.
88. Dong Z.M., Chapman S.M., Brown A.A., Frenette P.S., Hynes R.O. and Wagner D.D., The combined role of P- and E-selectins in atherosclerosis. J. Clin. Invest. 102: 145–152, 1998.
89. Frenette P.S. and Wagner D.D., Insights into selectin function from knockout mice. Thromb. Haemost. 78: 60–64, 1997.
90. Johnson R.C., Chapman S.M., Dong Z.M., Ordovas J.M., Mayadas T.N., Herz J., Hynes R.O., Schaefer E.J. and Wagner D.D., Absence of P-selectin delays fatty streak formation in mice. J. Clin. Invest. 99: 1037–1043, 1997.
91. Cybulsky M.I. and Gimbrone M.A. Jr., Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 251: 788–791, 1991.
92. Li H., Cybulsky M.I., Gimbrone M.A. Jr and Libby P., An atherogenic diet rapidly induces VCAM-1, a cytokine regulatable mononuclear leukocyte adhesion molecule, in rabbit endothelium. Arterioscler. Thromb. 1993(. 13) 197–204, 1991.
93. Iiyama K., Hajra L., Iiyama M., Li H., Di Chiara M., Medoff B.D. and Cybulsky M.I., Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. Circ. Res. 85: 199–207, 1999.
94. Gurtner G.C., Davis V., Li H., McCoy M.J., Sharpe A. and Cybulsky M.I., Targeted disruption of the murine VCAM1 gene. Essential role of VCAM-1 in chorioallantoic fusion and placentation. Genes Dev. 9: 1–14, 1995.
95. Gu L., Okada Y., Clinton S.K., Gerard C., Sukhova G.K., Libby P. and Rollins B.J., Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low-density lipoprotein-deficient mice. Mol. Cell. 2: 275–281, 1998.
96. Cook-Mills J.M., Johnson J.D., Deem T.L., Ochi A., Wang L. and Zheng Y., Calcium mobilization and Rac1 activation are required for VCAM-1 (vascular cell adhesion molecule-1) stimulation of NADPH oxidase activity. Biochem. J. 378: 539–547, 2004.
97. Eriksson E.E., Mechanisms of leukocyte recruitment to atherosclerotic lesions: future prospects. Curr. Opin. Lipidol. 15: 553–558, 2004.
98. Witztum J.L. and Berliner J.A., Oxodized phospholipids and isoprostanes in atherosclerosis. Curr. Opin. Lipidol. 9: 441–448, 1998.
99. Gimbrone M.A. Jr., Nagel T. and Topper J.N., Biomechanical activation: an emerging paradigm in endothelial adhesion biology. J. Clin. Invest. 100: S61–65, 1997.
100. Collins T., Endothelial nuclear factor-kappa B and the initiation of the atherosclerotic lesion. Lab. Invest. 68: 499–508, 1993.
101. Boring L., Gosling J., Cleary M. and Charo I.F., Decreased lesion formation in CCR2-/-mice reveals a role for chemokines in the initiation of atherosclerosis. Nature 394: 894–897, 1998.
102. Watson A.D., Leitinger N., Navab M., Faull K.F., Horkko S., Witztum J.L., Palinski W., Schwenke D., Salomon R.G., Sha W., Subbanagounder G., Fogelman A.M. and Berliner J.A., Structural identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interactions and evidence for their presence *in vivo*. J. Biol. Chem. 272: 13597–13607, 1997.
103. Wheeler A.P. and Ridley A.J., Why three Rho proteins? RhoA, RhoB, RhoC, and cell motility. Exp. Cell Res. 301: 43–49, 2004.
104. Van Aelst L. and D'Souza-Schorey C., Rho GTPases and signaling networks. Genes Dev. 11: 2295–2322, 1997.
105. Scita G., Tenca P., Frittoli E., Tocchetti A., Innocenti M., Giardina G. and Di Fiore P.P., Signaling from Ras to Rac and beyond: not just a matter of GEFs. EMBO J. 19: 2393–2398, 2000.
106. Danesh F.R., Sadeghi M.M., Amro N., Philips C., Zeng L., Lin S., Sahai A. and Kanwar Y.S., 3-Hydroxy-3-methylglutaryl CoA reductase inhibitors prevent high glucose-induced proliferation of mesangial cells via modulation of Rho GTPase/ p21 signaling pathway: Implications for diabetic nephropathy. Proc. Natl. Acad. Sci. USA 99: 8301–8305, 2002 (Epub 2002 Jun 04).
107. Eto M., Kozai T., Cosentino F., Joch H. and Luscher T.F., Statin prevents tissue factor expression in human endothelial cells: role of Rho/Rho-kinase and Akt pathways. Circulation 105: 1756–1759, 2002.
108. Endres M. and Laufs U., Effects of statins on endothelium and signaling mechanisms. Stroke 35: 2708–2711, 2004.
109. Ruiz-Velasco N. and Dominguez A., Vega.Statins upregulate CD36 expression in human monocytes, an effect strengthened when combined with PPAR-gamma ligands Putative contribution of Rho GTPases in statin-induced CD36 expression. Biochem. Pharmacol. 67: 303–313, 2004.

110. Bhattacharya M., Babwah A.V. and Ferguson S.S., Small GTP-binding protein-coupled receptors. Biochem. Soc. Trans. 32: 1040–1044, 2004.
111. Laufs U. and Liao J.K., Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. J. Biol. Chem. 273: 24266–24271, 1998.
112. Laufs U., Endres M., Stagliano N., Amin-Hanjani S., Chui D.S., Yang S.X., Simoncini T., Yamada M., Rabkin E., Allen P.G., Huang P.L., Bohm M., Schoen F.J., Moskowitz M.A. and Liao J.K., Neuroprotection mediated by changes in the endothelial actin cytoskeleton. J. Clin. Invest. 106: 15–24, 2000.
113. Goldstein J.L. and Brown M.S., Regulation of the mevalonate pathway. Nature 343: 425–430, 1990.
114. Park H.J., Kong D., Iruela-Arispe L., Begley U., Tang D. and Galper J.B., 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors interfere with angiogenesis by inhibiting the geranylgeranylation of RhoA. Circ. Res. 91: 143–150, 2002.
115. Wassmann S., Laufs U., Baumer A.T., Muller K., Konkol C., Sauer H., Bohm M. and Nickenig G., Inhibition of geranylgeranylation reduces angiotensin II-mediated free radical production in vascular smooth muscle cells: involvement of angiotensin AT1 receptor expression and Rac1 GTPase. Mol. Pharmacol. 59: 646–654, 2001.
116. Wagner A.H., Kohler T., Ruckschloss U., Just I. and Hecker M., Improvement of nitric oxide-dependent vasodilatation by HMG-CoA reductase inhibitors through attenuation of endothelial superoxide anion formation. Arterioscler. Thromb. Vasc. Biol. 20: 61–69, 2000.
117. Gorlach A., Brandes R.P., Nguyen K., Amidi M., Dehghani F. and Busse R., A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. Circ. Res. 87: 26–32, 2000.
118. Ishibashi T., Nagata K., Ohkawara H., Sakamoto T., Yokoyama K., Shindo J., Sugimoto K., Sakurada S., Takuwa Y., Teramoto T. and Maruyama Y., Inhibition of Rho/Rho-kinase signaling downregulates plasminogen activator inhibitor-1 synthesis in cultured human monocytes. Biochim. Biophys. Acta. 1590: 123–130, 2002.
119. Marrero M.B., Venema V.J., Ju H., He H., Liang H., Caldwell R.B. and Venema R.C., Endothelial nitric oxide synthase interactions with G-protein-coupled receptors. Biochem. J. 343: 335–340, 1999.
120. Tsutsui M., Shimokawa H., Tanaka S., Kuwaoka I., Hase K., Nogami N., Nakanishi K. and Okamatsu S., Endothelial Gi protein in human coronary arteries. Eur. Heart J. 15: 1261–1266, 1994.
121. Yang C.M., Chien C.S., Hsiao L.D., Pan S.L., Wang C.C., Chiu C.T. and Lin C.C., Mitogenic effect of oxidized low-density lipoprotein on vascular smooth muscle cells mediated by activation of Ras/Raf/MEK/MAPK pathway. Br. J. Pharmacol. 132: 1531–1541, 2001.
122. Yang C.M., Chiu C.T., Wang C.C., Chien C.S., Hsiao L.D., Lin C.C., Tu M.T. and Pan S.L., Activation of mitogen-activated protein kinase by oxidized low-density lipoprotein in canine cultured vascular smooth muscle cells. Cell Signal. 12: 205–214, 2000.
123. Zhao D., Letterman J. and Schreiber B.M., β-Migrating very low density lipoprotein (βVLDL) activates smooth muscle cell mitogen-activated protein (MAP) kinase via G protein-coupled receptor-mediated transactivation of the epidermal growth factor (EGF) receptor. J. Biol. Chem. 276: 30579–30588, 2001.
124. Uittenbogaard A. and Smart E.J., Palmitoylation of caveolin-1 is required for cholesterol binding, chaperone complex formation, and rapid transport of cholesterol to caveolae. J. Biol. Chem. 275: 255595–255599, 2000.
125. Everson W.V. and Smart E.J., Influence of caveolin, cholesterol and lipoproteins on nitric oxide synthase. Implications for vascular disease. Trends Cardiovasc. Med. 11: 246–250, 2001.
126. Uittenbogaard A., Ying Y. and Smart E.J., Characterization of a cytosolic heat-shock protein-caveolin chaperone complex. Involvement in cholesterol trafficking. J. Biol. Chem. 273: 6525–6532, 1998.
127. Stein O. and Stein Y., Atheroprotective mechanisms of HDL. Atherosclerosis 144: 285–303, 1999.
128. Von Eckardstein A. and Assmann G., Prevention of coronary heart disease by raising of high density lipoprotein cholesterol? Curr. Opin. Lipidol. 11: 627–637, 2000.
129. Toikka J.O., Ahotupa M., Viikari J.S., Niinikoski H., Taskinen M., Irjala K., Hartiala J.J. and Raitakari O.T., Constantly low HDL-cholesterol concentration relates to endothelial dysfunction and increased *in vivo* LDL-oxidation in healthy young men. Atherosclerosis 147: 133–138, 1999.
130. Krause B.R. and Auerbach B.J., Reverse cholesterol transport and future pharmacological approaches to the treatment of atherosclerosis. Curr. Opin. Investig. Drugs 2: 375–381, 2001.
131. von Eckardstein A., Nofer J.R. and Assmann G., High density lipoproteins and arteriosclerosis. Role of cholesterol efflux and reverse cholesterol transport. Arterioscler. Thromb. Vasc. Biol. 21: 13–27, 2001.
132. Fazio S., Increased atherogenesis in mice reconstituted with apolipoprotein E null macrophages. Proc. Natl. Acad. Sci. USA 94: 4647–4652, 1997.
133. Ohgami N., Nagai R., Miyazaki A., Ikemoto M., Arai H., Horiuchi S. and Nakayama H., Scavenger receptor class B type-I-mediated reverse cholesterol transport is inhibited by advanced glycation end products. J. Biol. Chem. 276: 13348–13355, 2001.
134. Wade D.P. and Owen J.S., Regulation of the cholesterol efflux gene, ABCA1. Lancet 357: 161–163, 2001.
135. Wang N., Silver D.L., Thiele C. and Tall A.R., ATP-binding cassette transporter A1 (ABCA1) functions as a cholesterol efflux regulatory protein. J. Biol. Chem. 276: 23742–23747, 2001.
136. Frank P.G., Galbiati F., Volonte D., Razani B., Cohen D.E., Marcel Y.L. and Lisanti M.P., Influence of caveolin-1 on cellular cholesterol efflux mediated by high-density lipoproteins. Am. J. Physiol. Cell Physiol. 280: C1204–C1214, 2001.
137. Oliver W.R. Jr, Shenk J.L., Snaith M.R., Russell C.S., Plunket K.D., Bodkin N.L., Lewis M.C., Winegar D.A., Sznaidman M.L., Lambert M.H., Xu H.E., Sternbach D.D., Kliewer S.A., Hansen B.C. and Willson T.M., A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. Proc. Natl. Acad. Sci. USA 98: 5306–5311, 2001.
138. Fazio S. and Linton M.F., The inflamed plaque. Cytokine production and cellular cholesterol balance in the vessel wall. Am. J. Cardiol. 88: 12E–15E, 2001.
139. Yamashita S., Hirano K., Sakai N. and Matsuzawa Y., Molecular biology and pathophysiological aspects of

plasma cholesteryl ester transfer protein. Biochim. Biophys. Acta 1529: 257–275, 2000.
140. Krieger M., Charting the fate of the good cholesterol. Identification and characterization of the high density lipoprotein receptor SR-BI. Ann. Rev. Biochem. 68: 523–558, 1999.
141. Trigatti B., Rigotti A. and Krieger M., The role of high-density lipoprotein receptor SR-BI in cholesterol metabolism. Curr. Opin. Lipidol. 11: 123–132, 2000.
142. Curtiss L.K. and Boisvert W.A., Apolipoprotein E and atherosclerosis. Curr. Opin. Lipidol. 11: 243–251, 2000.
143. Tall A.R., Jiang X.C., Luo Y. and Silver D., George Lyman Duff Memorial Lecture. Lipid transfer proteins, HDL metabolism and atherogenesis. Arterioscler. Thromb. Vasc. Biol. 20: 1185–1188, 2000.
144. Cohen J., Vega G.L. and Grundy S.M., Hepatic lipase. New insights from genetic and metabolic studies. Curr. Opin. Lipidol. 10: 259–268, 1999.
145. Thuren T., Hepatic lipase and HDL metabolism. Curr. Opin. Lipidol. 11: 277–284, 2000.
146. Rader D. and Jaye M., Endothelial lipase. A new member of the triglyceride lipase gene family. Curr. Opin. Lipidol. 11: 141–148, 2000.
147. VonEckardstein A., Huang Y. and Assmann G., Physiological role and clinical relevance of high-density lipoprotein subclasses. Curr. Opin. Lipidol. 5: 404–416, 1994.
148. Barrans A., Jaspard B., Barbaras R., Chap H., Perret B. and Collet X., Pre-β HDL. Structure and metabolism. Biochim. Biophys. Acta 1300: 73–85, 1996.
149. Fielding C. and Fielding P.E., Molecular physiology of reverse cholesterol transport. J. Lipid. Res. 36: 211–228, 1995.
150. Moestrup S.K. and Kozyraki R., Cubilin, a high density lipoprotein receptor. Curr. Opin. Lipidol. 11: 133–140, 2000.
151. Kozyraki R., Fyfe J., Kristiansen M., Gerdes C., Jacobsen C., Cui S.Y., Christensen E.I., Aminoff M., de la Chapelle A., Krahe R. *et al.*, The intrinsic factor-vitamin B-12 receptor, cubilin, is a high-affinity apolipoprotein A-I receptor facilitating endocytosis of high-density lipoprotein. Nat. Med. 5: 656–661, 1999.
152. Hammad S.M., Steffansson S., Twal W.O., Drake C.J., Fleming P., Remaley A., Brewer H.B. Jr and Argraves W.S., Cubilin, the endocytic receptor for intrinsic factor-vitamin B (12) complex, mediates high-density lipoprotein holoparticle endocytosis. Proc. Natl. Acad. Sci. USA 96: 10158–10163, 1999.
153. Dhaliwal B.S. and Steinbrecher U.P., Cholesterol delivered to macrophages by oxidized low density lipoprotein is sequestered in lysosomes and fails to efflux normally. J. Lipid. Res. 41: 1658–1665, 2000.
154. Aviram M. and Rosenblat M., Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. Free Radic. Biol. Med. 37: 1304–1316, 2004.
155. Becker A.E., de Boer O.J. and van der Wal A.C., The role of inflammation and infection in coronary artery disease. Ann. Rev. Med. 52: 289–297, 2001.
156. Vander Wal A.C., Piek J.J., de Boer O.J., Koch K.T., Teeling P., van der Loos C.M. and Becker A.E., Recent activation of the plaque immune response in coronary lesions underlying acute coronary syndromes. Heart 80: 14–18, 1998.
157. Libby P., Changing concepts of atherogenesis. J. Int. Med. 247: 349–358, 2000.
158. Libby P., Molecular bases of the acute coronary syndromes. Circulation 91: 2844–2850, 1995.
159. Herman M.P., Sukhova G.K., Kisiel W., Foster D., Kehry M.R., Libby P. and Schonbeck U., Tissue factor pathway inhibitor-2 is a novel inhibitor of matrix metalloproteinases with implications for atherosclerosis. J. Clin. Invest. 107: 1117–1126, 2001.
160. Mach F., Schonbeck U., Bonnefoy J.Y., Pober J.S. and Libby P., Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD-40. induction of collagenase, stromelysin and tissue factor. Circulation 96: 396–399, 1997.
161. Schonbeck U., Mach F., Sukhova G.K., Herman M., Graber P., Kehry M.R. and Libby P., CD40 ligation induces tissue factor expression in human vascular smooth muscle cells. Am. J. Pathol. 156: 7–14, 2000.
162. Marx N., Mackman N., Schonbeck U., Yilmaz N., Hombach V.V., Libby P. and Plutzky J., PPARalpha activators inhibit tissue factor expression and activity in human monocytes. Circulation 103: 213–219, 2001.
163. Yusuf S., Dagenais G., Pogue J., Bosch J. and Sleight P., Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N. Engl. J. Med. 342: 154–160, 2000.
164. Heart Protection Study Collaborative Group., MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 360: 23–33, 2002.
165. Ricciarelli R., Zingg J.M. and Azzi A., Vitamin E. Protective role of a Janus molecule. FASEB J. 15: 2314–2325, 2001.
166. Heinecke J.W., Is the emperor wearing clothes? Clinical trials of vitamin E and the LDL oxidation hypothesis. Arterioscler. Thromb. Vasc. Biol. 21: 1261–1264, 2001.
167. Keaney J.F. Jr., Atherosclerosis. From lesion formation to plaque activation and endothelial dysfunction. Mol. Aspects. Med. 21: 99–166, 2000.
168. Libby P., Current concepts of the pathogenesis of the acute coronary syndromes. Circulation 104: 365–372, 2001.
169. Jessup W., Kritharides L. and Stocker R., Lipid oxidation in atherogenesis: an overview. Biochem. Soc. Trans. 32: 134–138, 2004.
170. Stocker R. and Keaney J.F. Jr., Role of oxidative modifications in atherosclerosis. Physiol. Rev. 84: 1381–1478, 2004.
171. Vaughan C.J., Gotto A.M. and Basson C.T., The evolving role of statins in the management of atherosclerosis. J. Am. Coll. Cardiol. 35: 1–10, 2000.
172. Puddu P., Puddu G.M. and Muscari A., Current thinking in statin therapy. Acta Cardiol. 56: 225–231, 2001.
173. Puddu P., Puddu G.M. and Muscari A., HMG-CoA reductase inhibitors. Is the endothelium the main target?. Cardiology 95: 9–13, 2001.
174. Ambrosi P., Villani P., Habib G. and Bouvenot G., The statins: new properties. Therapie 58: 15–21, 2003.
175. Parissis J., Korovesis S., Kalivas P., Giagitzoglou E. and Katritsis D., Short-term atorvastatin therapy reduces monocyte-related inflammatory markers in the serum of hypercholesterolaemic patients. Eur. Heart J. 21: 154, 2000.

176. Chase A.J. and Newby A.C., Cerivastatin inhibits metalloproteinase production by human vascular smooth muscle cells in response to inflammatory stimuli, a possible mechanism for plaque stabillisation. Eur. Heart J. 21: 152, 2000.
177. Sinkiewicz W., Hoffman A., Blazejewski J., Bujak R., Budzynski J., Zbikowska M. and Palgan K., The serum immunoglobulin IgE, interleukin-4 and soluble form receptor CD23 dynamic changes in patients with hypercholesterolaemia II B treated with pravastatin. Eur. Heart J. 21: 155, 2000.
178. Paul A., Calleja L., Camps J., Osada J., Vilella E., Ferré N., Mayayo E. and Joven J., The continuous administration of aspirin attenuates atherosclerosis in apolipoprotein E-deficient mice. Life Sci. 68: 457–465, 2000.
179. Fuster V., Dyken M.L., Vokonas P.S. and Hennekens C., Aspirin as a therapeutic agent in cardiovascular disease. Circulation 87: 659–675, 1993.
180. Jukema J.W., Matching treatment to the genetic basis of (lipid) disorder in patients with coronary artery disease. Heart 82: 126–127, 1999.