Molecular biology of atherosclerosis

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Summary

The traditional view of atherosclerosis as a pathological lipid deposition within the artery wall has been redefined by a more complex concept of an ongoing inflammatory disease.
The atherosclerotic process is initiated when cardiovascular risk factors, through a chemical, mechanical or immunological insult, activate and/or injury the endothelium, thus contributing to endothelial dysfunction and fragmentation. This triggers a cascade of inflammatory reactions, in which monocytes, macrophages, T lymphocytes, vascular smooth muscle cells actively participate. Particularly, atherosclerotic lesions have been seen to have increased expression of T helper-1 cells together with increased levels of the T helper-1 related pro-inflammatory cytokines. Along with pro-inflammatory cytokines, other molecular factors involved in atherosclerosis appearance, progression and complication include chemokines, growth factors, vasoactive substances, enzymes, apoptosis signals and many others. Many of these molecular factors are both involved as possible markers of the atherosclerotic disease activity and burden, but may also play a crucial role in the pathogenesis of the disease. In recent years, the discovery of progenitor cells of myeloid origin has offered the prospect of merging the most recent theories on the pathogenesis of atherosclerosis with the evolving concept of a role of these progenitor cells in the repair of the injured vessel wall and the neovascularisation of ischemic tissues. This review summarizes current knowledge about the biology of atherosclerosis with emphasis on the mechanisms of endothelial damage and repair and on the concept that the turnover and replacement of endothelial cells is a major determinant in the maintenance of vascular integrity.

KEY WORDS: lipoproteins, inflammation, endothelial dysfunction, microparticles, progenitors.

Introduction

Atherosclerosis is a multifactorial disease that involves chronic inflammation from initiation to progression (1, 2), and all its risk factors contribute to atherosclerosis pathogenesis by aggravating the underlying inflammatory process (1, 2).

Molecular determinants of atherosclerosis appearance, progression and complication are numerous and very often overlap, in that the same factors that contribute to atherosclerosis initiation may also have a crucial role in the plaque growth and rupture. Molecular risk factors, like elevated plasma lipids and glucose levels, represent major risk factors for atherosclerosis and cardiovascular disease (3, 4); the latter risk factors have been implicated in atherosclerosis appearance and progression by starting a cascade of molecular events leading to plaque instability and cardiovascular events (1, 2). Even non-molecular risk factors, like hypertension, aging, smoking, through the action of molecular intermediates of the inflammation-oxidation balance, are major contributors to the pathogenesis of atherosclerosis. Along with cardiovascular risk factors, many other molecular players have a role in the atherosclerotic process. Inflammatory cytokines (1, 2), markers of oxidative burden (5), growth factors (6, 7), apoptosis signals (8), mediators of vascular tone (9), have all a pivotal influence on atherosclerosis evolution.
The appreciation of the role of all these molecular mediators of the atherosclerotic disease provides a mechanistic framework to understand more deeply about the atherosclerosis epidemic and the clinical benefits of newer therapeutic strategies in reducing the burden of atherosclerosis complications.

Lipids and atherosclerosis initiation and progression

The atherosclerotic process is initiated when lipid-containing lipoproteins accumulate in the intima and activate the endothelium (1, 2) (Figure 1). Thereafter, an inflammatory response occurs, which is characterised by recruitment of circulating leukocytes and the production of growth factors which encourage cell migration and proliferation (1, 2).
Studies in animal models showed that arterial retention of lipoproteins is regulated by the balance of delivery versus efflux (10-12). Delivery appears to be dependent on lipoprotein concentration, lipoprotein size, and the integrity of the endothelium. Particularly, delivery increases for higher plasma lipid levels and for smaller lipoproteins, like LDL, small dense LDL and triglyceride-rich lipoprotein remnants. All lipoproteins, apart from nascent triglyceride rich lipoproteins, penetrate and infiltrate the arterial wall (10, 11, 13, 14).
It has been also observed a significant inverse relation between lipoprotein diameter and fractional loss from the intima (15). The fractional loss of lipoproteins from the intima may be a combination of efflux of macromolecules from the intima-inner media into the vascular lumen and degradation of lipoproteins by the cells in the intima-inner media. It has been also observed (16) that the lipoprotein fractional loss may be also due to lipoproteins that were irreversibly attached to arterial wall components, most likely glycosaminoglycans.
The infiltration and retention of lipoproteins in the arterial intima initiate an inflammatory response in the artery wall (17, 18). Indeed, modification of retained lipoproteins contribute to the release of phospholipids that can activate endothelium (18). That is the basis for increased expression of adhesion molecules and inflammatory genes by endothelial cells and for the loss of

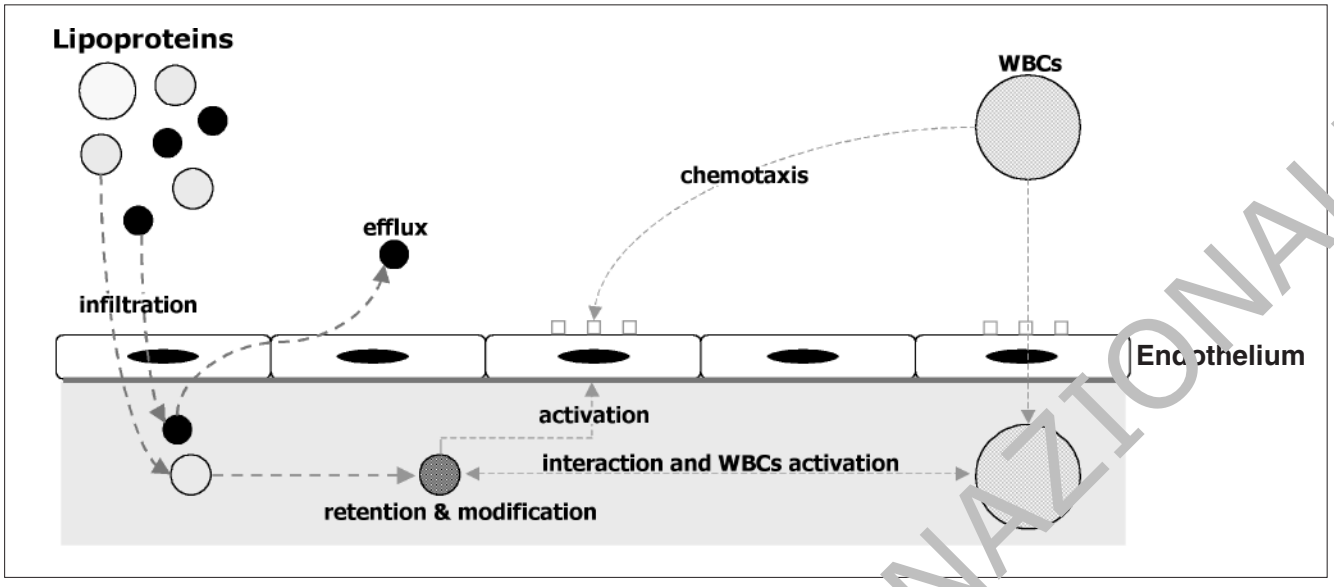

Figure 1 - Mechanisms of lipoprotein mediated atherogenesis. Intermediate size lipoproteins (grey circles) are those that are preferentially retained into the artery wall and activate the atherosclerosis cascade. Small size lipoproteins (black circles) infiltrate the artery wall, but may be also cleared away from the artery. WBCs: white blood cells.

the morpho-functional integrity of the endothelium, that is also named endothelial dysfunction.

Endothelial dysfunction is today believed one of the most important initial steps of the atherosclerosis process (19), and most cardiovascular risk factors, including dyslipidemia, have been implicated in its appearance (20). We have found that hypercholesterolemic as well as hypertriglyceridemic patients have an impaired endothelial function that may be partially reversed by statin or fibrate therapy (21, 22). We also found that oxidized LDL may have a role in impairing endothelial function (23). More recently, we have demonstrated that patients with primary hypercholesterolemia have an increased endothelial damage, as demonstrated by an elevated plasma concentration of endothelial microparticles (24). These data strongly suggest that dyslipidemias contribute to a significant extent to endothelial damage and initiation of the atherosclerosis process.

Other than endothelial dysfunction, early atherosclerosis is characterized by reduced large artery distensibility.

A few studies (25, 26) have reported conflicting data on the influence of high plasma cholesterol levels on reduced arterial elasticity, a novel marker of early atherosclerosis and premature coronary heart disease risk (27). Some studies found patients with hypercholesterolemia have stiffer blood vessels than matched controls (26) and that cholesterol reduction may have a role in reducing large artery stiffness (28). Other authors reported an increased aortic distensibility in subjects with heterozygous familial hypercholesterolemia (25), an inverse association between cholesterol and aortic stiffness (29), as well as unchanged or increased arterial stiffness after cholesterol-lowering therapy (30, 31). We demonstrated that despite a role for hypercholesterolemia to favour arterial stiffening, inflammation seem to play a major influence on arterial distensibility regardless of other cardiovascular risk factors (32). Interestingly, both short-term low-cholesterol/low-saturated fat diet (33) and statin therapy (28) in hypercholesterolaemia may be effective in improving large artery stiffness, most likely through the reduction of plasma cholesterol levels but also through the mitigation of low-grade systemic inflammation.

Inflammation: a major mediator of atherosclerosis

The accumulation of lipids within the artery wall may initiate an inflammatory process in the artery. However, other major risk factors have been observed to contribute to the activation of a low-grade systemic inflammatory reaction (1, 2). Diabetes, smoking, hypertension, hypoalphalipoproteinemia and other risk factors have been associated with increased plasma levels of several biomarkers of inflammation (34-37). Interestingly, all these risk factors may contribute to endothelial activation and dysfunction (Figure 2). Activated endothelial cells express on their luminal surface leukocyte adhesion molecules, which cause white blood cells adhesion on the vascular surface at sites of activation. Once the white blood cells have attached, chemokines produced in the underlying intima stimulate them to migrate into the subendothelial space (1, 2). Monocytes entered the intima differentiate into macrophages and express at high level scavenger receptors and toll-like receptors. Scav-

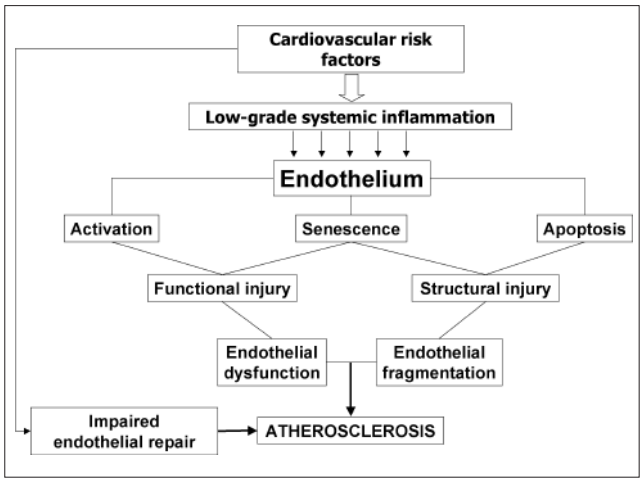

Figure 2 - The role of endothelial injury and repair in the history of atherosclerosis.

enger receptors may contribute to internalize apoptotic cell fragments, oxidized LDL particles and other detrites. Lipid deposition into macrophages contribute to foam cell formation. Toll-like receptors can initiate a signal cascade that leads to macrophage activation and production of inflammatory cytokines, proteases, and cytotoxic radical molecules (1, 2). Also T-lymphocytes infiltrate the atherosclerotic lesions. These T-cells recognize antigens presented to them by activated macrophages. Activated T cells therefore differentiate mainly into T-helper 1 cells and begin producing interferon-gamma, which in turn increases the process of antigen presentation by macrophages to lymphocytes and stimulates synthesis of other cytokines like tumor necrosis factor and interleukin-1. All these cytokines stimulate the production of many other inflammatory and cytotoxic molecules thus increasing the burden of the inflammatory reaction.

The demonstration that all these events linked to the inflammation process within the artery may contribute to atherosclerosis development come from several lines of evidence.

First, inflammatory cells are present in atherosclerotic lesions at all stages of development, exhibit activation markers and are particularly prominent at sites of plaque rupture (38). Second, adoptive transfer of purified CD4+ T cells from oxLDL-immunized mice accelerates atherosclerosis (39) and the absence of CD4+ cells in apoE KO mice leads to reduced atherosclerosis, indicating that CD4+ cells constitute a major proatherogenic cell population (40). Third, patients with clinically relevant inflammatory diseases like rheumatoid arthritis develop early atherosclerosis (41-43). Fourth, even low-grade systemic inflammation may be associated with premature atherosclerosis development (32,33). Finally, several markers of inflammation have been prospectively associated with an increased risk of the most typical complications of atherosclerosis, including ischemic heart disease events (44, 45).

We have found that young to middle aged patients with rheumatoid arthritis with low disease activity, free from cardiovascular risk factors and overt cardiovascular disease, have an altered endothelial reactivity that seems to be primarily related to the disease associated chronic inflammatory condition (41). Circulating CD4+CD28(null) lymphocytes are increased in rheumatoid arthritis (46). Patients with persistent CD4+CD28(null) cell expansion show preclinical atherosclerotic changes, including arterial endothelial dysfunction and carotid artery wall thickening, more significantly than patients without expansion (42). These findings suggest a contribution of this cell subset in atheroma development in rheumatoid arthritis.

Although rheumatoid arthritis represent an independent risk factors for atherosclerosis, this may be confounded by continuous pharmacologic treatment. Primary Sjögren's syndrome shares several features of this disease and therefore represents an interesting model for verifying the presence of accelerated atherosclerosis in the absence of pharmacologic interference. We found that subclinical atherosclerosis was evident in about one-half of the patients with Sjögren's syndrome (43). Its association with some features typical of connective tissue diseases, such as the presence of anti-SSA, suggests that the immune dysregulation characterizing this autoimmune disorder may play a key role in inducing early atherosclerosis (43).

Interestingly, in all these clinical settings caractherized by an exaggerated activation of the inflammation cascade, an association between humoral markers of inflammation and vascular damage has been found. The same correlation has been often found in other settings which are caractherized by a low-grade inflammatory cascade activation. Accordingly, increased plasma C-reactive protein (CRP) and inflammatory cytokine levels have been associated with endothelial dysfunction, arterial stiffness and arterial intima-media thickness in patients carrying one or more cardiovascular risk factors. However, it is important to underline that, even in apparently healthy subjects, the presence of elevated plasma levels of acute phase proteins (i.e. CRP, fibrinogen) and cytokines (i.e. interleukin-6) is associated with an increased risk of future cardiovascular events (44, 45). We have found that plasma CRP levels may provide independent information on ischemic heart disease risk mainly in middle-aged men and in the case of ischemic heart disease events that occur relatively soon after the baseline evaluation (44). It was also found that elevated plasma interleukin-6 concentrations are more strongly related to ischemic heart disease risk than CRP and fibrinogen (45). An inflammation score based on high plasma interleukin-6 and fibrinogen levels used in combination with traditional risk factors may improve our ability to adequately identify high risk individuals (45).

Mechanisms of vascular damage and repair

It is widely believed that cardiovascular risk factors promote atherogenesis by damaging endothelium (Figure 2). Endothelial status has been mainly assessed by focusing the attention on the quantification of the endothelium capacity to modulate arterial vasomotion (47). An alternative way to get information on endothelial health is to measure products of endothelial cell injury. Quantification of circulating endothelial cells has been suggested as a method of assessing endothelial damage, given that exposure of the endothelium to most cardiovascular risk factors may cause the detachment of endothelial cells from the intimal monolayer, thus releasing mature endothelial cells in peripheral blood (48). More recently, there has been considerable interest in a novel marker of endothelial cell injury, namely endothelial microparticles (EMPs) (49, 24). Microparticles are small vesicles released from the membrane surface during cell activation, injury, or apoptosis, and display the typical surface cell proteins and cytoplasmic components of their cell origin. Endothelial cell vesiculation happens also under physiologic condition, possibly as a mechanism of endothelial cell renewal or cross-talk with other cellular targets. Elevated levels of EMPs, mostly defined as CD31+/CD42- MPs, are found in patients with a variety of vascular diseases and in subjects exposed to cardiovascular risk factors (24). In the setting of hypercholesterolemia, we had previously found that the number of circulating CD31+/CD42- microparticles was associated with aortic stiffness and that microparticles from hypercholesterolemic patients cause a significant impairment of endothelial repair in vitro (24).

Under physiological conditions the integrity of the endothelial monolayer is maintained by replication of adjacent cells (50); however, in conditions of increased endothelial injury, regeneration of the injured endothelium may be assisted by endothelial progenitor cells (EPCs) homing into the artery wall (50). Evidence that EPCs contribute to endothelial cell regeneration comes from animal studies and computer-based simulation models in humans (51,52). In hypercholesterolemic apolipoprotein E knock-out mice, the systemic transfusion of EPCs significantly improved endothelial dysfunction (51), whereas in humans, an EPCs homing rate of 5% per year was sufficient to significantly delay defects in endothelial integrity (52).

Progenitors to vascular endothelial cells mainly reside in the adult bone marrow, from where they can be mobilized into circulation by cytokines and growth factor signals. EPCs are defined by the expression of antigens indicating staminality, like CD133 and CD34, but also antigens that are typical of mature endothelial cells, like VEGFR-2 or KDR (53). These progenitors are involved in the process of repair of ischemic organs but also in the repair of the injured endothelium. The endothelial repair is a highly coordinated multi-step process that requires EPCs mobilization into circulation, their migration in the vascular endotheli-

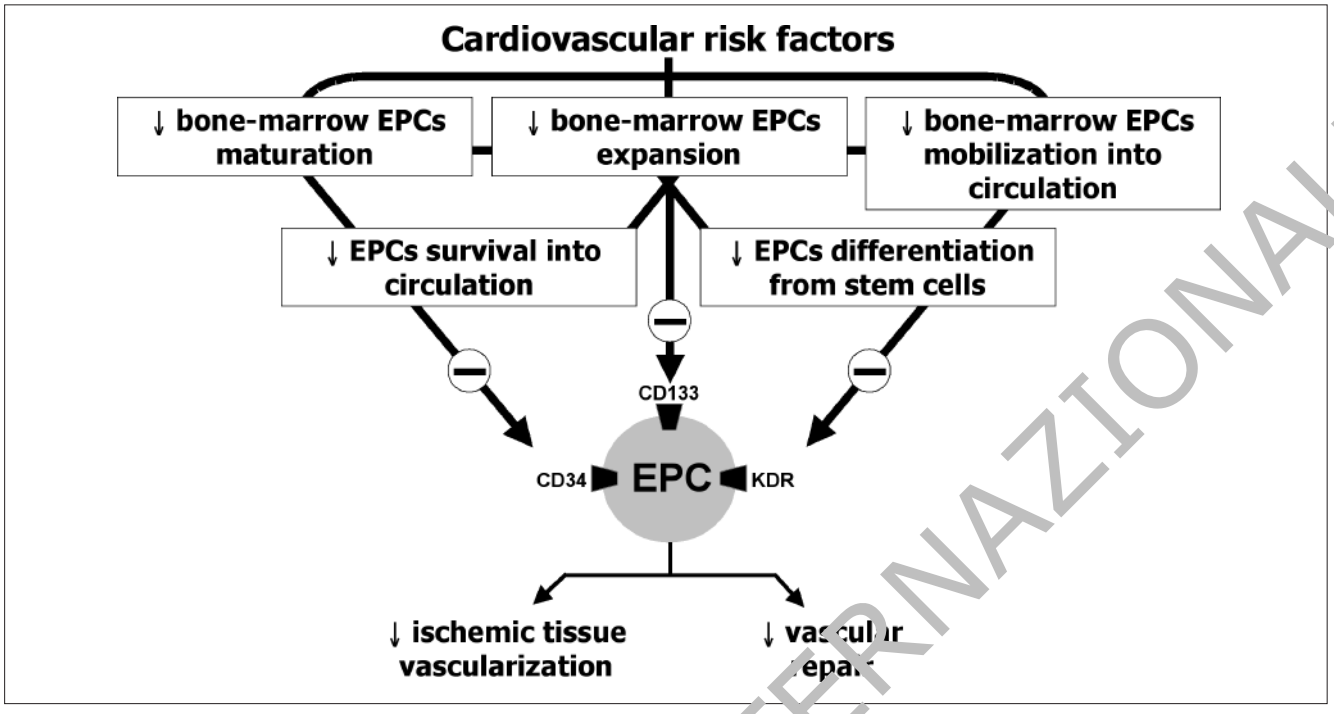

Figure 3 - Causes of endothelial progenitor cell loss, defective neovascularization of ischemic tissues and impaired vascular repair. EPCs: endothelial progenitor cells.

um and finally their differentiation into mature and healthy endothelial cells. Since it is very common to find a significant endothelial injury in subjects with cardiovascular risk factors, it is extremely important to know whether there is a relationship between cardiovascular risk factors and the number of circulating endothelial progenitor cells (Figure 3). In this respect, we recently found that hypercholesterolemic patients have a reduced number of circulating EPCs compared to normolipidemic subjects (24); and the same was also found in hypertensive patients compared to healthy normotensive controls (54). However, not only dyslipidemia and hypertension are typically characterized by a reduced number of endothelial progenitors, but also other major risk factors are today known to cause a significant EPCs loss, thus reducing their potential to repair ischemic organs and the injured endothelium. So, it is important to know why a reduced number of endothelial progenitor cells is commonly found in subjects at increased cardiovascular risk. Today we know that most cardiovascular risk factors may contribute to reduce the number of circulating EPCs (Figure 3) by reducing:

a. their initial maturation, expansion and mobilization from the bone marrow;
b. their vitality and survival in the blood.

We found that, likewise mature endothelial cells, also EPCs may be mechanically and functionally injured, thus releasing microparticles (55). Particularly, we demonstrated that cultured EPCs undergo extensive apoptosis and release a significant amount of microparticles when exposed to a range of concentrations of the pro-apoptotic hydrogen-peroxide. In addition, we found in human blood CD34+/KDR+ MPs, possibly indicating that EPCs may be injured in the circulation, expecially in patients at increased cardiovascular risk, and may consequently release MPs in vivo. Finally, we also found that, likewise microparticles from mature endothelial cells, also microparticles from circulating EPCs may have an active role in the process of vascular damage (55).

Although the new paradigm of risk factors induced EPCs loss is now deeply ingrained in the scientific community, an understanding of how some risk factors may contribute to the loss of circulating EPCs remains only partially understood. Homeobox genes encode for transcription factors, which regulate cell proliferation and migration and play an important role in the development of the cardiovascular system during embryogenesis (56, 57); these genes were also involved in a differentiation-like process occurring in normal adult cells and act as regulator genes that maintain tissue or organ specificity in the adult body (56, 57). Homeobox A9 (HOXA9) is a member of the homeobox gene family and a number of possible target genes of HOXA9 in human CD34+ cells were recently identified (58). By modulating downstrem target genes HOXA9 contributes in physiological conditions to endothelial commitment of EPCs, post-natal neovascularization and injured endothelium repair (59-61). HOXA9 overexpression is associated with an increased number of EPCs, while in HOXA9 deficient mice there is a lower number of EPCs and impaired postnatal neovascularization after ischemia (61). We found that downregulation of HOXA9 expression in peripheral CD34+ cells may have a role in the loss of circulating EPCs, thus potentially impairing postnatal neovascularization and vascular repair.

Conclusions

The lesions of atherosclerosis represent a series of highly specific cellular and molecular responses. This multi-step process of vascular injury under cardiovascular risk exposure, activation of local and systemic inflammation, vascular cells senescence and apoptosis and contribution of progenitor cells to vascular repair is higly regulated by key molecular signals. Thus, translating this molecular knowledge to interventional cardiovascular medicine, such a detailed understanding in the complex regulation of atherosclerosis development and progression may be helpful for more effectively preventing the many clinical consequences which parallel the complication of vulnerable atherosclerotic plaques.

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