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The autoimmune concept of atherosclerosis

Cecilia Grundtman and **Georg Wick**
Laboratory of Autoimmunity, Division for Experimental Pathophysiology and Immunology, Biocenter, Innsbruck Medical University, Innsbruck, Austria

Abstract

Purpose of review—This review summarizes the recent data on the 'Autoimmune Concept of Atherosclerosis', according to which the first stage of this disease is due to an autoimmune reaction against arterial endothelial cells expressing heat shock protein 60 (HSP60) and adhesion molecules when stressed by classical atherosclerosis risk factors. Special emphasis is put on oxidized low-density lipoproteins as early endothelial stressors.

Recent findings—Plasma cholesterol and LDL levels considered 'normal' by the medical community are possibly too high from an evolutionary viewpoint. The proinflammatory milieu at sites of early atherosclerotic lesions could be conducive to oxidation of LDL *in situ*. LDL oxidation can also take place at nonvascular sites or in the circulation under general proinflammatory conditions explaining its proatherosclerotic role in 'normocholesterolemic' individuals.

Summary—We hypothesize that the plasma cholesterol and LDL levels currently considered normal are evolutionarily too high. Cholesterol and/or oxidized low-density lipoprotein, even as a mild HSP60-inducing endothelial stressor, function as a ubiquitous risk factor. If this hypothesis is true, most members of developed societies might be at risk to develop atherosclerotic plaques at anti-HSP60-immunity-triggered intimal inflammatory foci, irrespective of the primary risk-factor(s).

Keywords

atherosclerosis; cholesterol; classical atherosclerosis risk factors; heat shock protein; oxidized low-density lipoprotein; vascular-associated dendritic cells

Introduction

Inflammatory processes have been demonstrated pathohistologically in advanced atherosclerotic lesions (plaques). This review focuses on the incipient inflammatory stage of atherogenesis. In early lesions, activated T cells are the first mononuclear cells invading the arterial intima [1-3], which is already populated with a network of dendritic cells [4]. Next, monocytes (macrophages *in situ*), a few B cells, and finally vascular smooth muscle cells (SMCs) [1] immigrate into the intima. A depletion of T or B cells leads to an attenuation of atherosclerosis [5,6••,7]. However, T cells seem to be essential for atherosclerosis development, whereas B cells and antibodies play an accelerating and perpetuating role. In late lesions, macrophages, together with vascular SMCs, represent the most abundant

Correspondence to Cecilia Grundtman, PhD, Laboratory of Autoimmunity, Division of Experimental Pathophysiology and Immunology, Biocenter, Innsbruck Medical University, Peter Mayr Strasse 4a, A-6020 Innsbruck, Austria, Tel: +43 512 9003 70967; fax: +43 512 9003 73960; Cecilia.Grundtman@i-med.ac.at.

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subpopulation by far thus exceeding T-cell numbers [1,3]. Although intra-lesional fibroblasts and extracellular matrix (ECM) components, especially by formation of the fibrous lesional cap, contribute to plaque stability, the progressive proinflammatory conditions also lead to upregulation of matrix metalloproteinase (MMP) expression and concomitant downregulation of tissue inhibitors of metalloproteinases (TIMPs), culminating in plaque rupture [8-10]. These inflammatory processes are the basis of a vicious cycle mediated by the release of increasing quantities of proinflammatory cytokines and chemokines, promoting the influx of additional inflammatory cells into the intima. Quantitative and qualitative deficiency of T regulatory cells (Tregs) seems to significantly contribute to intralesional T effector (Teff) cells getting out of control [11,12•].

With respect to innate immunity, triggering by microbial components, for example, via binding to Toll-like receptors (TLRs), and activation of signaling via the MyD88–IRAK pathway, emerged as the most likely atherogenic mechanism [13,14,15••,16•]. In addition, TLR stimulation promotes the accumulation of lipids in macrophages and consequently foam cell formation [17-19]. With respect to adaptive immunity, biochemically altered oxidized low-density lipoproteins (oxLDLs), as well as heat shock protein 60 (HSP60) might be responsible for direct immune stimulation of Teffs or immune-complex deposition in atherosclerotic plaques [20].

The autoimmune concept of atherosclerosis

The ‘Autoimmune Hypothesis of Atherosclerosis’, according to which the earliest stage of atherosclerosis is due to an autoimmune reaction against HSP60, was first formulated in 1992 [21]. It was then just a hypothesis based on our experimental and clinical findings [21]. Today, based on a wealth of data supporting the hypothesis, one can rightly speak of the ‘Autoimmune *Concept* of Atherosclerosis’, recently discussed in great detail [22••]. The ‘Autoimmune Concept of Atherosclerosis’ is summarized in Fig. 1. The core of this concept is that an HSP60-induced inflammatory process initiates atherosclerosis, and all other events, including the formation of foam cells, which occur later.

According to in-vitro and in-vivo findings, the incipient inflammatory stage of atherosclerosis results from various classical atherosclerosis risk factors. All classical risk factors lead to the simultaneous surface expression of HSP60 and adhesion molecules on endothelial cells and thus are early endothelial stressors. Figure 2 is a schematic representation of the HSP60-inducing potential of various atherosclerosis risk factors expressed as approximately fold abundance of expression at RNA and protein levels. Arterial endothelial cells, lifelong ‘prestressed’ by the higher arterial blood pressure and flow conditions, are more susceptible to stress induced by different risk factors than venous endothelial cells [23,24]. Venous bypass conduits subjected to arterial blood pressure and flow conditions also develop atherosclerosis-like lesions and finally restenosis [23,24]. Recently, this also has been shown in an aortic valve regurgitation model [25]. Synthesis of proinflammatory cytokines, chemokines, and growth factors also contribute significantly to endothelial cell dysfunction. Human endothelial cells exposed to increased laminar shear stress display a specific change in behavior and modified gene expression, most probably entailing atheroprotective effects [26•].

Vascular-associated dendritic cells

Antigen presentation is a key event in adaptive immune response. Dendritic cells, professional antigen-presenting cells (APCs), induce primary and secondary immune responses. Resident vascular-associated dendritic cells (VADCs) are present within the tertiary lymphoid structures in the aortic adventitia of atherosclerotic vessels [27•]. These might represent a unique subpopulation of dendritic cells, with distinct ultrastructural

features including a unique tubulovesicular system. Ultrastructural investigations of normal arterial intima and early atherosclerotic lesions reveal two main populations of VADCs – type I and type II [28,29]. These become increasingly heterogeneous during lesion development [30]. In atherosclerotic lesions, dendritic cells often form clusters with T cells, suggesting that direct activation of dendritic cells might occur within the arterial wall [30]. Accumulation of dendritic cells in arterial lesions is associated with plaque growth and inflammation [31]. Dendritic cells are irregularly distributed in atherosclerotic plaques, with the highest frequency in areas of neovascularization in the shoulder of the plaque. Interestingly, not all foci of plaque neovascularization contain large numbers of lymphocytes but all areas of neovascularization consistently contain VADCs [30].

Newly arrived dendritic cells and dendritic cell precursors invading the aortic lesion might come not only from the circulation but also from the adventitia via *vasa vasorum*. Dendritic cells may easily migrate from *vasa vasorum* to capillaries [32,33], and ingrowth of *vasa vasorum* together with the lymphatics to advanced atherosclerotic plaques might facilitate the exchange of dendritic cell precursors and maturing dendritic cells between the arterial tissue and lymph [29,30]. We have shown earlier that the intima of healthy human babies, children, and young adults harbors an accumulation of mononuclear cells exactly at those sites subjected to hemodynamic stress (turbulent flow) that tend to develop atherosclerotic lesions later in life. In analogy to the mucosa-associated lymphoid tissue (MALT), we designated these accumulations as vascular-associated lymphoid tissue (VALT) [3,4,34,35]. VALT consists of Langerhans-like VADCs, T cells, resident macrophages, and scarce mast cells distributed throughout the subendothelial layer of the arterial intima of *normocholesterolemic* humans, rabbits, and wild type C57BL/6J mice [3,4,36,37]. In the arterial wall, VADCs may have a surveillance role, and by priming T cells and cross-presenting these antigens to T cells, they may facilitate tolerization against autoantigens [38-40]. Prolonged contact of blood with the arterial wall in areas exposed to turbulent flow compared to areas subjected to laminar shear stress enable VADCs to capture potentially harmful exogenous or autoantigens and present these to T cells. T cells entering the intima may also be tolerized against arterial antigens presented by VADCs [27•,41].

Scavenger receptors, expressed on dendritic cells, can mediate oxLDL uptake and induce a proinflammatory cytokine profile triggering dendritic cell maturation and differentiation [42]. Intimal lipids can also be taken up by resident intimal dendritic cells [43••]. Mice receiving malondialdehyde-modified LDL (MDA-LDL) pulsed myeloid dendritic cells show more extensive atherosclerotic lesions than controls, with increased inflammatory hallmarks and antigen-specific immune responses [44]. Deficiency of APC invariant chain (CD74) reduces atherosclerosis in mice and impairs adaptive immune response to ‘endogenous’ disease-specific antigens (MDA-LDL and HSP60) [45]. In the absence of $CD11c^+$ cells, there was a striking 55% reduction in early lipid accumulation in the aortic wall [46]. Accumulated lipid was found only in extracellular spaces, apparently ignored by circulating monocytes. These data suggest that, under hypercholesterolemic conditions, VADCs can regulate the accumulation of lipid in the earliest stages of plaque formation and are central in the atherosclerosis process because of their direct effect on both cholesterol homeostasis and immune responses [47]. Whether VADCs can capture circulating native and/or oxidized LDL that may interact with the endothelium and thereby collecting much of the earliest cholesterol that accumulates in the arterial intima is presently unclear.

Tolerogenic dendritic cells have recently been shown to be useful in treating autoimmune disorders and improving allograft survival. Tolerogenic dendritic cells pulsed with native ApoB100 reduce the autoimmune response against LDL and attenuate atherosclerosis [48••]. Furthermore, vaccination using oxLDL-pulsed mature dendritic cells effectively reduces atherosclerotic lesion formation, inducing oxLDL-specific T cells, and enhancing

the levels of anti-oxLDL IgG [49]. The therapeutic use of dendritic cell-pulsed antigen(s) to induce a specific tolerogenic response is limited, but encouraging.

Circulating levels of oxidized low-density lipoproteins

Many authors believe that LDL oxidation does not take place in the circulation, and therefore it must occur in the subendothelial space of the arterial wall. However, small quantities of lipoprotein with many of the characteristics of oxLDL have been found circulating in human plasma [50] with higher levels in atherosclerotic patients than healthy controls [51]. No correlation was found between clinical symptoms and plasma oxLDL levels, indicating its pathogenic role in the early stages of the disease. This is also supported by the fact that plasma oxLDL levels are elevated prior to atherosclerosis progression [52]. Furthermore, the reduction in plasma oxLDL levels coincided with lesion development and oxLDL accumulation in the atherosclerotic intima [53], suggesting that plasma oxLDL can migrate between the intimal regions and circulation. Increased levels of oxLDL are present in human gingival crevicular fluid compared to plasma of healthy individuals [54], indicating that oxLDL could be generated in inflamed extra-arterial tissues, transferred to the circulation, rapidly taken up into the arterial wall, and contribute to the perpetuation of atherosclerosis.

The presence of circulating antibodies against oxLDL suggests its availability as an antigen also outside the vascular system [55]. A variety of oxidized lipid products also have been identified in plasma, and degradation products of some of these have even been demonstrated in urine, with increased levels in atherosclerosis patients [56]. The source of these compounds is not clear. Cholesterol crystals, detected not only in the necrotic cores but also in the subendothelial areas in early atherosclerotic settings, trigger inflammasome activation both in humans and in mouse, leading to interleukin-1β secretion [57 ••,58]. Their possible source may be the circulation.

Oxidized LDL in early atherosclerotic lesions

LDL can be oxidized enzymatically, nonenzymatically by transition metal ions, and by other catalysts, triggering both innate and adaptive immunity. The process of LDL oxidation is assumed to occur in two main steps. Minimally oxLDL (mmLDL), which retains its affinity to the LDL receptor, activates antiapoptotic signaling and induces inflammatory changes. The recruitment of inflammatory cells may result in the production of a large variety of cytokines and chemokines that could continue the oxidation process of LDL [59]. With further LDL lipid oxidization, and LDL protein modification, loss of recognition by the LDL receptor and a shift to recognition by scavenger receptors occur, leading to foam cell formation, predominantly in anti-inflammatory (M2) macrophages. As a result, activated M2 macrophages shift to a proinflammatory profile [60•]. A variety of macrophage phenotypes have been found in plaques, which may all have different impacts on facets of plaque development [61]. The modified forms of LDL are more proatherogenic than native LDL. Mucosal immunizations with native LDL peptides show atheroprotective effects [62]. Previously, no T cells were believed to react with native LDL components. New data show that peripheral T cells in atherosclerotic mice recognize peptide motifs of native LDL particles and ApoB100 and, surprisingly, oxidation extinguishes rather than promotes LDL-dependent T-cell activation [63••]. oxLDL-reactive T cells can be localized in plaques, lymph nodes, and in the plasma of atherosclerosis patients and experimental animals. oxLDL has been demonstrated to precede accumulation of monocytes and formation of fatty streaks [64]. Several mechanisms for increased monocyte recruitment attributable to the presence of modified LDL in the arterial wall have been identified. Modified LDL can enhance monocyte adhesion by inducing the expression of adhesion molecules, chemotactic,

and growth factors in endothelial cells, and also activate platelets, with increased platelet-monocyte aggregation, and monocyte adherence to the endothelium [65,66•,67]. The major pathways in the early lesion development are summarized in Fig. 3.

Thus, although there is substantial evidence that oxidized forms of LDL are produced in the arteries of mice as well as men, definite data to establish that the lipids that generate foam cells derive from these oxidized lipoproteins, as opposed to native, aggregated, or non-oxidatively modified forms of LDL, are not yet available.

Moreover, if an increased oxLDL level is such an important – if not the most important – risk factor for the initiation and progression of the disease, why do the majority of patients display normal cholesterol serum levels?

Why is cholesterol such an important atherosclerosis risk factor?

In more than 60% of patients with clinical symptoms of atherosclerosis, total blood cholesterol levels and LDL levels are within the range currently considered to be ‘normal’, namely, 200 mg/dl or less for total cholesterol and 100 mg/dl or less for LDL cholesterol. Concentrations of native LDL in extracts of human intimal samples typically exceed 100 mg/dl. There are notable examples in which the primary atherogenetic role of high LDL cholesterol levels is obvious, such as cases of human heterozygous or homozygous familial hypercholesterolemia due to mutations of the LDL receptor. However, this condition does not reflect the ‘poor man’s’ atherosclerosis that to a large extent afflicts normocholesterolemic patients.

An explanation for the accumulation of LDL in atherosclerotic lesions of normocholesterolemic patients is still lacking. Our hypothesis is that cholesterol levels of human beings considered ‘normocholesterolemic’ in terms of current medical criteria are still significantly hypercholesterolemic from an evolutionary viewpoint. We are equipped with a stone-age genome, but live in times where cultural evolution has far outpaced biological evolution. Populations following a more ‘Western’ life-style, for instance, show relatively high serum cholesterol/LDL concentrations and a significantly higher rate of atherosclerosis than those adhering to a Mediterranean lifestyle regarding nutrition and physical exercise [68,69]. Recent imaging studies of 4000-year-old Egyptian mummies revealed the presence of atherosclerotic lesions in some of these suggesting that atherosclerosis is not exclusively a modern disease [70-72]. However, embalming and thus preservation of bodies, organs, and vessels was a privilege of affluent segments of these societies and do not reflect the situation of the malnourished majority enduring hard physical labor and probably displaying very low cholesterol/LDL levels. Also, after World Wars I and II, prisoners returning home after years of severe hunger combined with exhausting labor suffered from many diseases, but not atherosclerosis [73]. Furthermore, statins can reduce the incidence of major cardiovascular events in healthy persons without hypercholesterolemia (LDL cholesterol levels of ≤130 mg/dl) but with elevated high-sensitive C-reactive protein levels. These effects were consistent in all sub-groups evaluated, including those customarily considered to be at low risk (LDL cholesterol levels of ≤100 mg/dl) [74]. In addition, both statins and aspirin have been shown to have anti-inflammatory properties [74-76].

We hypothesize that a plethora of different risk factors can initially act as endothelial cell stressors and lead to the first inflammatory-immunological stage of atherosclerosis. In persons currently considered ‘normocholesterolemic’, these create local conditions that foster the influx of native and/or oxLDL also from the circulation. Plasma cholesterol or modified LDL levels are emerging as major risk factors in practically all clinical studies, as they are too high from the evolutionary point of view, act as primary endothelial cell

stressors alone or together with other risk factors, and make an essential contribution to lesion formation proceeding from early nonfoam cell-dominated inflammatory lesions to fatty streaks and atheromas in 'normocholesterolemic' persons.

Conclusion

Our hypothesis is that from an evolutionary viewpoint, plasma cholesterol levels currently considered 'normal' are far too high in the majority of humans. Cholesterol – even as a relatively mild adhesion molecule and HSP60-inducing endothelial cell stressor – is a ubiquitous risk factor. If true, irrespective of their primary risk factor(s), most members of developed societies are at risk of developing fatty streaks and even atherosclerotic plaques, because of the presence of intimal inflammatory foci arising from pre-existing anti-HSP60 immunity.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p 417).

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Key points

- All classical atherosclerosis risk factors can act as endothelial stressors provoking the simultaneous surface expression of HSP60 and adhesion molecules at known arterial predilection sites.
- HSP60 can then act as a ‘danger signal’ for the immune system recognized by pre-existing innate and adaptive anti-HSP60 immunity.
- T cells predominate over macrophages in early atherosclerotic lesions. An increased number of vascular-associated dendritic cells can be found in areas of hemodynamic stress and they may play a role in early tolerization against autoantigens.
- oxLDL exert relatively low levels of stress on endothelial cell but are nevertheless the most common risk factor for atherosclerosis. oxLDL lead to foam cell formation at sites of primary inflammation/immunological reaction.
- The importance of cholesterol and oxLDL in atherogenesis may be because of the fact that from an evolutionary viewpoint, people currently considered ‘normocholesterolemic’ are in fact hypercholesterolemic.

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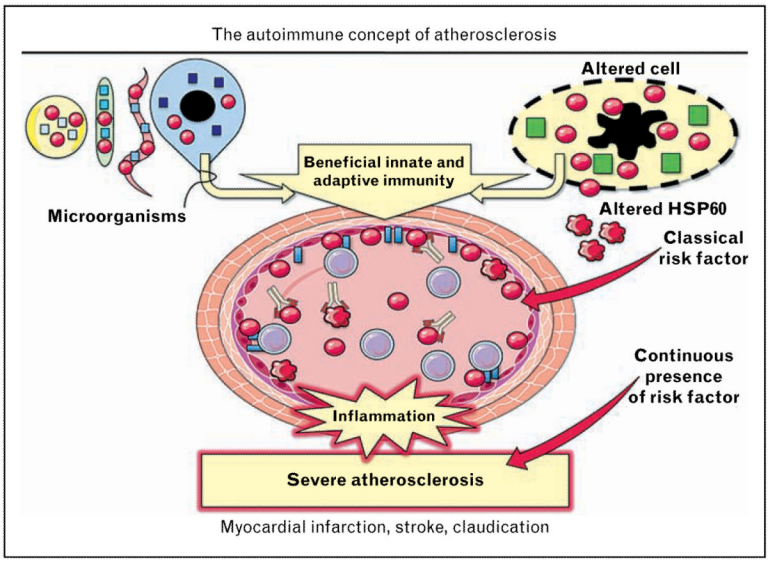

Figure 1. The 'autoimmune concept of atherosclerosis' is based on the well proven fact that all healthy human beings develop protective immunity against microbial (bacterial and parasitic) heat shock protein 60 as well as *bona fide* physiological autoimmunity against biochemically modified autologous HSP60 produced by and released from stressed and/or disintegrated cells

Heat shock protein 60 (HSP60) of various bacterial species displays over 95% sequence homology, and there is over 50% homology at the DNA and protein levels between prokaryotic and eukaryotic (including human) HSP60. When arterial endothelial cells are stressed by classical atherosclerosis risk factors, they simultaneously express HSP60 and adhesion molecules (ICAM-1, VCAM-1, and ELAM-1) on their surface, making them target cells for pre-existing beneficial cellular and humoral anti-HSP60 immunity. HSP60-reactive T cells invade the arterial intima and initiate the first inflammatory stage of atherosclerosis, which is followed by monocytes/macrophages and vascular smooth muscle cells. Anti-hHSP60 (auto)antibodies accelerate and perpetuate the disease. This first inflammatory stage of atherosclerosis is still reversible. However, in the continued presence of risk factors, severe advanced atherosclerosis (plaques) develops with deleterious consequences, such as myocardial infarction, stroke, and claudication. Thus, atherosclerosis is 'the price we pay for pre-existing anti-HSP60 immunity' if we maltreat our arteries by exposing them to classical risk factors. , HSP60; , altered HSP60; , cell-specific proteins; , adhesion molecules; , T cell; , anti-hHSP60 antibody. Partly adapted from Servier Medical Art.

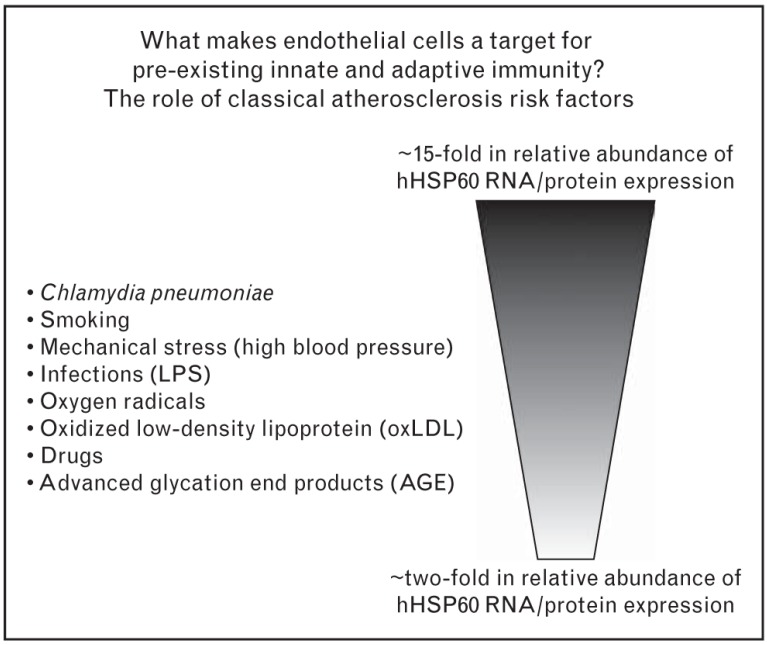

Figure 2. All classical atherosclerosis risk factors studied in our laboratory so far act as endothelial cell stressors leading to the simultaneous surface expression of heat shock protein 60 and adhesion molecules (ICAM-1, VCAM-1, and ELAM-1)

An approximate ranking of the respective stressor potential on protein and/or RNA levels is given. All data in the figure relate to the in-vitro treatment of human umbilical vein endothelial cells (HUVECS). The invivo effects of mechanical stress and bacterial lipopolysaccharide (LPS) have been shown in rats and rabbits, respectively. In several instances, comparative results were obtained with human arterial vs. venous endothelial cells showing a higher susceptibility of the former for the stressor effect of risk factors.

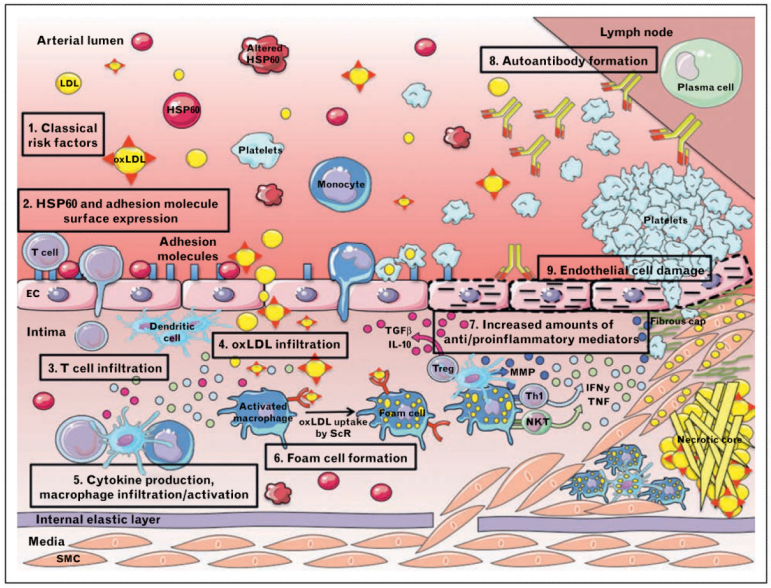

Figure 3. Compared with venous endothelial cells, arterial endothelial cells are exposed to higher mechanical stress because of the arterial blood pressure and flow conditions

This is especially true at branching points subjected to an increased turbulent hemodynamic shear stress predisposing these sites for the development of atherosclerotic lesions. (1–2) Classical atherosclerosis risk factors first act as endothelial stressors leading to the simultaneous expression of heat shock protein 60 (HSP60) and adhesion molecules. (3) HSP60-reactive T cells adhere to stressed endothelial target cells and transmigrate into the intima with its pre-existing network of Langerhans-like dendritic cells that act as antigen-processing and antigen-presenting cells similar to the situation in the skin. Whether sensitization of T cells attracted to these sites occurs *in situ* or in the draining lymph nodes remains to be seen. (4–6) Whether LDL oxidation occurs already in the circulation or in the mononuclear cell infiltrate in the intima by oxidative stress is still a matter of debate. It is, however, well proven that oxLDL itself acts as an endothelial stressor leading to HSP60 expression. Furthermore, activated platelets can bind LDL in the circulation, adhere to endothelial cells, and mediate monocyte attachment. Monocytes (loaded or not with LDL) can then migrate into the primary T-cell-dominated inflammatory intima where they can differentiate into macrophages. In a further step, smooth muscle cells (SMCs) from the media are attracted into the intima. Macrophages, dendritic cells, and vascular SMCs possess scavenger receptors, which enable them to take up oxLDL and transform into foam cells. (7) T cells initiate the disease, and local immune reaction creates a microenvironment with an altered balance of pro-inflammatory and anti-inflammatory mediators. (8) (Auto)antibodies accelerate and perpetuate the disease. (9) Continuing presence of risk factors led to endothelial damage. Partly adapted from Servier Medical Art.