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REVIEW

Inflammation and Atherosclerosis

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Purpose. *The aim of this article is to discuss the role of inflammation in atherosclerosis.*
Summary. *An initial chemical, mechanical or immunological insult induces endothelial dysfunction. This triggers a cascade of inflammatory reactions, in which monocytes, macrophages, T lymphocytes and vascular smooth muscle cells participate. Leukocyte adhesion molecules, cytokines, growth factors and metalloproteinases participate in all stages of atherogenesis. Almost all of the traditional risk factors for atherosclerosis are associated with and participate in the inflammatory process. Many infectious agents, mainly* Chlamydia pneumoniae, *have been proposed as potential triggers of the cascade. The immune system has been implicated in plaque formation, through the activation of cellular and humoral immunity against innate or microbial heat shock protein 60. Methods of detection of systemic or local plaque inflammation have been developed and research is being conducted on the potential use of anti-inflammatory and antibiotic drugs in atherosclerosis.*

Keywords: Inflammation; Infection; Atherosclerosis; Endothelium; Risk factors; Plaque evolution.

Introduction

Almost 200 years ago London surgeon J. Hodgson observed the inflammatory characteristics of atherosclerotic lesions.[1] A causative role of inflammation and infection in the pathogenesis of atherosclerosis was first proposed in 1908 by Sir William Osler.[2] For the major part of the 20th Century, the lipid theory dominated the field of atherogenesis. R. Ross reopened the discussion on the inflammatory nature of atherosclerosis some 30 years ago, with his first significant review on atherosclerotic plaque formation.[3]

The response-to-injury theory of atherogenesis, is still valid with minor alterations. Endothelial dysfunction seems to be the first step in atherogenesis. From this point on, an inflammatory response is triggered that leads to the development of atherosclerotic plaque.[4] This review summarizes the experimental and clinical evidence that links inflammation to atherosclerosis.

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Endothelium: a Complex Organ in Health and Disease

It has been established in the last 15 years, that the endothelium is not a simple lining of cells on the inner arterial wall. Endothelial cells secrete a wide variety of active molecules[5] (Fig. 1). Healthy endothelium is an important barrier to the free passage of molecules and cells to the underlying interstitium and a dynamic endocrine organ, which not only mediates endothelium-dependent vasodilation, but also actively inhibits leukocyte adhesion and migration, platelet adhesion and aggregation and vascular smooth muscle cell proliferation and migration. It also inhibits coagulation, promotes fibrinolysis and participates actively in immune and inflammatory reactions.[6]

Endothelial dysfunction or activation can occur in response to a variety of stimuli such as oxidized LDL, free radicals caused by smoking, hypertension, diabetes, genetic alterations, elevated plasma homocysteine concentrations and infectious microorganisms. Endothelial homeostasis is disturbed and this affects permeability, vasoconstriction, coagulation and

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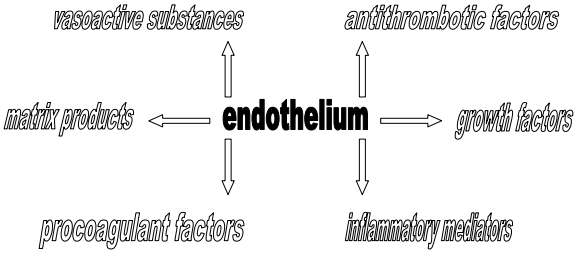

Fig. 1. The endocrine nature of the endothelium: endothelial cells are mini factories that produce a large variety of important substances, that control vasomotor activity (nitric oxide, endothelin and leukotrienes), coagulation (thromboplastin, thromboxane A_2, von Willebrand factor, factor V, platelet activating factor, plasminogen activator inhibitor, heparin, anti-thrombin, thrombomodulin, prostacyclin, plasminogen activator), vascular inflammation (interleukins, cytokines), extracellular matrix production (fibronectin, collagen, proteoglycanes) and cell hypertrophy (insulin like growth factor, colony stimulating factor).

triggers inflammatory and immunologic reactions. It has been demonstrated that endothelial dysfunction is one of the earliest signs of atherosclerosis, even in the absence of angiographic evidence of disease. Reduced nitric oxide (NO) activity is one of the earliest and more important markers of endothelial dysfunction.[7] Endothelin-1 (ET-1), a vasoconstrictor, seems to be in a sensitive balance with NO regulating vascular tone. It has been shown that ET-1 may be involved in atherosclerosis[8] and that ET receptors have an increased expression in human atherosclerotic plaques.[9] If the inflammatory response fails to neutralize the offender, the inflammation goes on and stimulates migration and proliferation of smooth muscle cells. The response is mediated by monocyte-derived macrophages and specific subtypes of T-lymphocytes. Excessive inflammatory and fibroproliferative responses lead to thickening of the arterial wall and the formation of the atherosclerotic lesion.

Inflammation in the Initiation of Atherosclerosis

Healthy endothelium, does not normally bind white blood cells. Soon after atherogenic diet is initiated, many endothelial cells begin to express on their surface adhesion molecules (selectins, intercellular adhesion molecules ICAMs, vascular cell adhesion molecule VCAMs) that act as receptors for glycoconjugates and integrins present on monocytes and T-cells. In particular VCAM-1, binds precisely the types of leukocytes found in early human and experimental atheroma, the monocyte and the T lymphocyte. Mice genetically engineered to express defective VCAM-1, show interrupted lesion development.[10]

Defective atheroprotective mechanisms, could also contribute to the initiation of atherosclerosis. At branch points of the arterial tree, the absence of normal shear stress reduces local production of endothelium-derived NO,[11] which can block the expression of VCAM-1.[12] Turbulence and abnormal shear stress, can increase the production of ICAM-1,[13] and promote the production by arterial smooth muscle cells of proteoglycans, which can bind and retain lipoprotein particles, which after their oxidation promote an inflammatory response at sites of plaque formation.[14]

After their attachment to the arterial wall, leukocytes begin their migration into the intima, with the help of several chemoattractant molecules. Monocyte chemoattractant protein-1 (MCP-1) appears to be responsible for the migration of monocytes into the intima. Another class of T cell chemoattractants helps lymphocytes penetrate the inner arterial wall.[15] Once inside the intima, monocytes are converted into macrophages and start to express on their surface, scavenger receptors for modified lipoproteins, under the influence of macrophage-colony stimulating factor (M-CSF).[16] M-CSF leads to the ingestion of lipids, and to the multiplication and differentiation of monocytes into macrophage foam cells. This characteristic lesion which consists of macrophage foam cells and T-cells, situated under a monolayer of endothelial cells, is the first lesion of atherosclerosis, the so called fatty-streak.

Inflammatory Evolution of Atherosclerosis

In the fatty-streak lesion, T-cells are activated and together with native vascular wall cells, secrete cytokines (tumor necrosis factor-β, γ-interferon), fibrogenic mediators and growth factors that can promote the migration and proliferation of smooth muscle cells (SMC) and the construction of a dense extracellular matrix around them, characteristic of an advanced atherosclerotic lesion. Medial SMCs express specialized enzymes that can degrade the elastin and collagen in response to inflammatory stimulation. This degradation of the arterial extracellular matrix permits the penetration of the SMCs through the internal elastic laminae and their migration to the subintimal area.[4] At the same time SMCs secrete factors that recruit additional monocytes.[17]

The macrophage-lipid component of the plaque, T-lymphocytes and the fibromuscular component (SMCs and extracellular matrix), enter a vicious cycle of cell migration to the subintimal area, cell proliferation and overproduction of fibrous tissue, leading to intimal thickening, intermediate lesions and

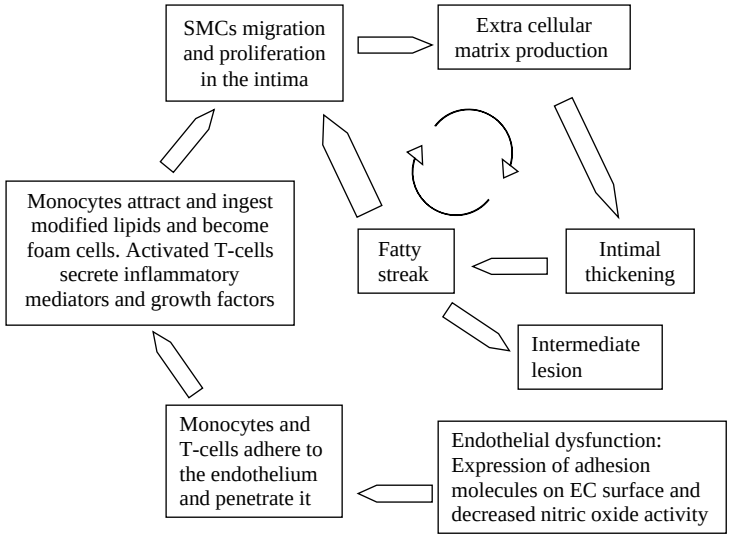

Fig. 2. Inflammatory cells (monocytes, T-cells) and proinflammatory mediators (cytokines, interleukins) have a key role in the initial phase and progression of atheroma formation. Repeated cycles of intimal thickening lead from the first lesion of atherosclerosis, the fatty streak, to intermediate and advanced lesions.

restructuring of the atheroma (Fig. 2). All three classes of activated cells release proinflammatory mediators. Inflammatory cytokines, including IL-1, tumor necrosis factor (TNF) and CRP induce the expression of cellular adhesion molecules, which mediate adhesion of the leukocytes to the endothelium.[18] Gradually, the so called advanced atherosclerotic lesion is formed, with the characteristic core of lipids and necrotic tissue which is covered by a fibrous cap. The necrotic core is formed from the apoptosis and necrosis of macrophages, which empty their lipid contents inside the plaque. The fibrous cap, is probably formed by migration of typical contractile vascular SMCs from the media, which accounts for the frequent medial wasting observed at the base of atherosclerotic plaques[19] and from collagen produced by the SMCs.

Involvement of Inflammation in Plaque Destabilization and Atherothrombotic Complications

Myocardial infarction (MI), fatal coronary thrombosis and stroke are the main consequences of either catastrophic rupture or surface erosion of the fibrous cap. Rupture is responsible for 80% of fatal MI in men. It occurs in regions of high tangential stress and collagen depletion.[20] Plaque erosion accounts for 50% of MI in young women.[20] In a quarter of cases, plaque does not rupture. The endothelium is simply replaced by the prothrombotic inflammatory cells.[21] Stable advanced lesions usually have uniformly dense fibrous caps. At autopsy, plaque rupture usually occurs in areas of sustained inflammation, macrophage accumulation and apoptosis,[22] often at the shoulders of the lesion.

Activated T-cells may stimulate MMP production by macrophages in the lesion.[23] The activated macrophage, produces these proteolytic enzymes, capable of degrading the collagen of the plaque's protective fibrous cap, rendering it susceptible to rupture. Activated T lymphocytes, produce γ-interferon that can halt collagen synthesis by the SMCs, limiting its capacity to renew the collagen that reinforces the cap.[24] The potentially dangerous lesions are often non-occlusive and thus difficult to diagnose by angiography. On histopathological examination, active inflammation is evident in these lesions. Half of all infarctions occur in arteries that have $<50\%$ luminal stenosis, that is generally considered a stenosis hemodynamically insignificant.[25] However, the presence of inflammation seems to be the key event. Plaque inflammation may be associated with increased plasma concentrations of both fibrinogen and CRP.[26]

Inflammation and Traditional Risk Factors of Atherosclerosis

Low density lipoproteins (LDLs) and especially their modified forms (oxLDL, glycLDL, etc.), have a deleterious effect on the endothelium and the underlying smooth muscle. Once LDLs are trapped in the intima, they undergo oxidative modification. These modified lipids can induce the expression of adhesion molecules, proinflammatory cytokines and other

mediators of inflammation in macrophages and endothelial cells. They are taken up by the macrophages and finally they are found in the necrotic core of the advanced lesion, after the apoptosis of the macrophages. Despite the abundant evidence supporting the importance of LDL oxidation in human atherosclerosis, anti-oxidant (β-carotene, vitamin C and vitamin E) therapy has not been shown to reduce cardiovascular event rate in clinical trials.[27]

Very low-density lipoproteins (VLDL) and intermediate density lipoproteins (IDL), can undergo oxidation just like LDL and some evidence even suggest that beta VLDL can activate inflammatory functions of vascular endothelial cells.[28] The atheroprotective effect of high-density lipoprotein (HDL) may in part result from its anti-inflammatory and antioxidant properties. HDL particles can carry antioxidant enzymes, such as platelet-activating factor, acetylhydrolase and paraoxonase, which breakdown oxidized lipids and neutralize their proinflammatory effects. Surprisingly, HDL isolated from coronary artery disease patients without diabetes and with average lipid levels, did not protect LDL from oxidation.[29]

Sphingosine kinase (SphK), an enzyme responsible for the conversion of sphingosine to sphingosine-1 phosphate, has emerged in recent years as an important link between lipid metabolism and inflammation in the arterial wall. The SphK pathway, responsible for the expression of adhesion molecules and cell proliferation of human SMC,[30] seems to be activated by cytokines and ox-LDL and inhibited by HDL.[31] Finally, there is now a considerable amount of evidence, that statins, the cornerstone of lipid lowering therapy, also have anti-inflammatory properties,[32] and part of their effect on cardiovascular mortality and morbidity is due to their anti-inflammatory action.

Hypertension is one of the classical risk factors for atherosclerosis. Angiotensin II (AII), the principal product of the renin-angiotensin system and a potent vasoconstrictor, increases protein synthesis and smooth muscle hypertrophy.[33] It also increases smooth muscle lipoxygenase activity, which in turn increases inflammation and LDL oxidation. AII augments the formation of hydrogen peroxide and free radicals such as superoxide anion and hydroxyl radicals in plasma.[34] These oxidative inflammatory products block the formation of nitric oxide by the endothelium, increase leukocyte adhesion and peripheral resistance. Arterial SMCs treated with AII demonstrate increased expression of proinflammatory cytokines such as IL-6 and monocyte chemoattracting protein-1 (MCP-1), while endothelial cells show increased expression of the adhesion molecule VCAM-1.[35] By interrupting the expression of adhesion molecules and cytokines, angiotensin-converting enzyme inhibitors (ACEI) exert anti-inflammatory effects on the development of atherosclerosis. The protective effect of Angiotensin II blockade goes over and beyond its anti-hypertensive action.[36]

Five major molecular mechanisms have been implicated in hyperglycemia induced endothelial damage: activation of protein kinase C isoforms via *de novo* synthesis of the lipid second messenger diacylglycerol, increased hexosamine pathway flux, increased advanced glycation end product (AGE) formation, increased polyol pathway flux and activation of the proinflammatory nuclear transcription factor nuclear factor-kappa B.[37] In an effort to evaluate diabetes-induced oxidative stress and inflammation, it has been documented that there is an increased cytokines, chemokines and adhesive molecules production in the arterial wall of streptozotocin-induced diabetic swine.[38] Oxidative stress was responsible for this inflammatory response mediated by AGE-induced activation of the transcriptional factor nuclear factor-kappa B in coronary adventitial fibroblasts providing a mechanistic link between diabetes, oxidative stress and inflammation. Inflammation can also extend into the tunica media, inducing atrophy and fibrosis. Increased content of macrophage-derived MMP-2 and -9 within the intima-media interface of advanced plaques has been described. This increased activity of MMP is associated with disruption of the internal elastic lamina, an independent predictor of plaque rupture.[39] Plaque composition may be different in patients with diabetes. Macrophage infiltration and thrombus formation are increased in advanced coronary plaques from diabetic patients with unstable angina.[40]

Adipose tissue can synthesize cytokines such as TNF-α and IL-6[41] and in this way obesity can promote inflammation and advance atherogenesis, independently of its effects on insulin resistance and lipoprotein metabolism. Peroxisome proliferators-activated receptors (PPARs) are lipid-activated nuclear receptors that serve as transcriptional regulators of genes encoding for proteins involved in glucose and lipid metabolism. In obese insulin-resistant mice, diet restriction caused a 45% weight loss, an upregulation of PPAR-alpha and PPAR-gamma and a change in the expression of genes regulating glucose transport and insulin sensitivity, lipid metabolism, oxidative stress, and inflammation, most of which are under the transcriptional control of these PPARs.[42] It has also been shown, that in overweight men, low fat and very low carbohydrate diet, results in significant decreases

in absolute concentrations of TNF-α, IL-6, CRP and ICAM-1.[43] In another study, MCP-1 production in isolated adipocytes, was reduced after weight loss in morbid obese subjects.[44] Therefore, it seems that there is an association between obesity and in particular visceral obesity and low grade inflammation.[45]

There is increasing evidence that major dietary patterns are associated with markers of inflammation and endothelial dysfunction. In a recent study, spontaneously hypertensive rats were fed broccoli sprouts, whose ingredient glucoraphanin has been shown to reduce oxidative stress. This diet decreased NF-κB activation, which resulted in decreased tissue infiltration of activated macrophages[46] and reduced blood pressure. In a non-human primate model, ovariectomized monkeys on a moderately atherogenic diet plus soy isoflavones or conjugated equine estrogens, were studied at the end of a 3-year period. Both isoflavones and equine estrogens reduced VCAM-1, but only estrogens reduced MCP-1.[47]

Postmenopausal women at risk for coronary artery disease, were placed on a rigorous high-fiber low-fat diet and on a regular exercise intervention program. This intervention not only improved their metabolic and lipid profiles, but also reduced the levels of inflammatory (CRP, SAA) and adhesion (ICAM-1) molecules without caloric restriction.[48] In a very important recent study, two types of diet were compared: the rich in fiber and white meat Mediterranean diet and the rich in saturated fat, carbohydrates and red meat Western diet. Mediterranean diet was inversely associated with CRP and E-selectin plasma concentrations, whereas Western diet was correlated with CRP, interleukin-6, E-selectin, ICAM-1 and VCAM-1 levels,[49] significant markers of inflammation and endothelial dysfunction.

Inflammation and Infection

In the light of the importance that inflammation has assumed in the recent years in the process of atherogenesis, many have suspected that an infectious agent could be behind chronic inflammation of atheroma. Both bacteria and viruses have been implicated. *In vitro* CMV infection of macrophages, increases secretion of IL-1, TNF-α and macrophage colony stimulating factor (MCSF).[50] Indeed it seems that CMV infection is more important for cardiovascular risk, when it elicits a persistent inflammatory response, evidenced by high CRP values.[51]

Escherichia coli endotoxin regulates IL-1 and TNF-α production in endothelial and vascular smooth muscle cells.[52] Cytokines produced by endotoxin activated cells, induce the expression of soluble adhesion molecules P-selectin, ICAM-1 and VCAM-1 in endothelial cells. Helicobacter pylori has been associated with coronary events in the West of Scotland study cohort.[53]

Chlamydia pneumoniae has been isolated from atherosclerotic plaques from almost all the sites of the arterial tree and has also been associated with the severity of the lesions and with acute clinical syndromes.[54] In chronic chlamydial infections, heat shock protein (Hsp) 60 is overproduced. It is also traced on the atheroma and it induces the expression of adhesion molecules[55] and the oxidation of LDL. Recently, there has been an effort to attribute plaque inflammation not to one but to multiple pathogens. This was termed 'pathogen burden' and it seems to have a stronger association with endothelial dysfunction, inflammation of the lesion and cardiovascular event rate.[56]

Inflammation and the Immune System

Inflammation of atheromatous lesions could result from an exaggerated immune response. In basic defense mechanisms, expression of scavenger receptors (SR) and toll-like receptors (TLR), are increased in monocytes and macrophages. SR induce endocytosis whereas TLR activate transcription factors, such as NF-κB. Activated NF-κB induces expression of genes that increase production of chemoattractant molecules, adhesion molecules, cytokines and reactive oxygen species.[57]

The more sophisticated adaptive immune response consists of macrophage presentation of the antigen to the T lymphocytes. Activated T lymphocytes, attack the antigen, stimulate B lymphocytes to secrete antibodies and enhance inflammation by releasing cytokines (TNF-α, INF-γ, IL-1). Cytokines induce production of chemoattractant molecules including MCP-1. Genetically modified mice lacking MCP-1 or its receptor, demonstrate a slower development of atheromas despite high lipid diet.[58]

Although the antigens serving as immune targets in atheroma are unknown, heat shock protein is a most likely candidate. Some authors have suggested, that while originally HSPs are intercellular proteins, marked stress-induced over expression may lead to their presentation on the cell surface, stimulating an autoimmune reaction and thereby contributing to the development of atherosclerosis. This conception of autoimmune atherogenesis, was strongly supported by the facts that increased expression of human Hsp60 has been observed in endothelial cells,

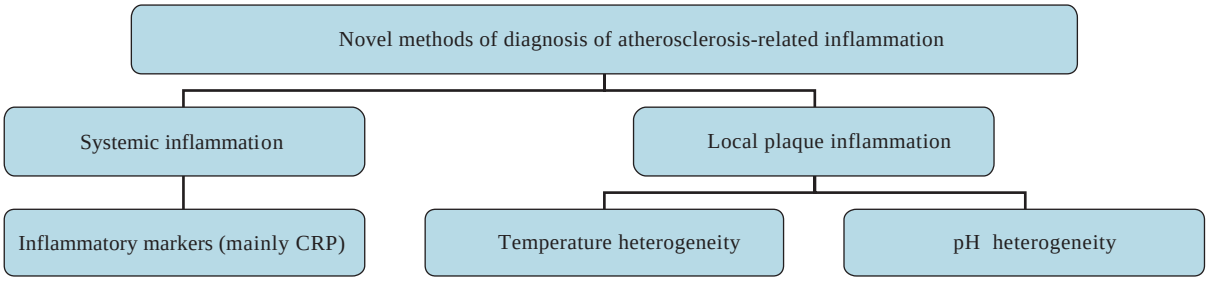

Fig. 3. Diagnostic methods of local and systemic inflammation in atherosclerosis. Many serum inflammatory markers have been implicated in atherogenesis. On the other hand, only quite recently temperature and pH of the plaque have been associated to local inflammation and clinical events. Inflammation as we know increases temperature and reduces pH.

macrophages and smooth muscle cells on human plaques and that anti-human Hsp60 antibodies correlate with both the presence and the extent of coronary artery disease.[59]

The Future

Various inflammatory mediators, including adhesion molecules, cytokines, chemoattractant and growth factors, have been shown to have distinct roles in the inflammation of atheroma.[60] In the last few years, there has been an increasing interest in specific serum markers that could reflect the severity of systemic inflammation. CRP, serum amyloid A (SAA), proinflammatory cytokines and adhesion molecules have all been linked to increased risk of future cardiovascular events, but the association was strongest for CRP. TNF-α and IL-6 have been found to be associated with 1-year mortality in critical limb ischemia patients.[61] New techniques have been devised in order to detect local inflammation in the plaque, by measuring temperature and pH heterogeneity[62] based on the rationale that inflammation increases temperature and reduces pH (Fig. 3).

The next step would be to treat inflammation and determine if there is any benefit in cardiovascular risk. Statins and ACE inhibitors have already shown, that besides their main action they also have anti-inflammatory properties and this is reflected on their impact on cardiovascular mortality. Old (aspirin) and new (COX-2 inhibitors, PPAR-γ agonists) anti-inflammatory drugs, are being increasingly used in studies in patients with vascular disease and the first evidence has been encouraging.[62] Unfortunately, the latest results have shown that more than one COX-2 inhibitors, are associated with an increased cardiovascular risk.[63] Also large antibiotic trials have shown no reduction in the rate of cardiovascular events.[64]

Conclusions

In the years to come, specific markers and anti-inflammatory agents may be used in everyday routine diagnosis and management of vascular disease. Results of experimental anti-inflammatory medication in cardiovascular disease, may further support the clinical importance of inflammation.

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