Thrombosis Journal

BioMed Central

Review

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Risk factors in coronary atherosclerosis athero-inflammation: the meeting point

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Published: 17 July 2003

Thrombosis Journal 2003, **1**:4

Received: 22 April 2003

Accepted: 17 July 2003

This article is available from: http://http//www.thrombosisjournal.com/content/1/1/4

Review

Following Russel Ross [1], there is now general agreement that vessel wall inflammation constitutes a major factor in the development of atherosclerosis, atheroma instability and plaque disruption followed by local thrombosis, that underlies the clinical presentation of acute coronary syndromes [2–4]. Endothelial-cell injury is the main stimulus for development of the atherosclerotic plaque: an inflammatory-fibroproliferative response results from various forms of insult to the endothelium.

Arterial endothelium changes rapidly in response to specific stimuli. Elevated and modified LDL, cigarette smoking, hypertension, diabetes mellitus, genetic alterations, increase of plasma homocysteine, and infectious microorganisms such as herpes virus or *Chlamydia pneumoniae*, were considered by Ross as possible causes of endothelial dysfunction [1]. In atherosclerosis and other diseases, dysfunctional vascular endothelium leads to leukocyte recruitment. The initial phase of inflammation is usually silent and the atherosclerosis preclinical window is fairly long. In altered arterial endothelium there is increased monocyte adhesion as well as impaired nitric oxide production and vascular relaxation [5]. Adherence of monocytes to the endothelial surface is facilitated by the expression of the adhesion molecules vascular cell adhesion molecule-1 (V-CAM 1) and intercellular adhesion molecule-1 (ICAM-1).

Endothelial function is a balance between vascular cell protectors and risk factors. Under physiological conditions, vascular endothelium has antithrombogenic potential. Activation of endothelia cells by proinflammatory cytokines or infectious agents is associated with a loss of antithrombotic properties. Endothelial dysfunction, injury and inflammation induce cell imbalance and a normal endothelium with anticoagulant properties becomes prothrombotic. Endothelial dysfunction is associated with a decrease of nitric oxide and an increase of oxidative stress, an important promotor of the inflammatory process [6,7] Risk factors, either acute (infection, immune local reaction) or permanent (hypertension, diabetes, dyslipemia, obesity, hyperhomocysteinemia, smoking, etc) induce endothelial dysfunction, cell injury, and a proinflammatory environment resulting in a local, tissue factor mediated activation of the clotting cascade [6,8,9]. In addition, it seems that expression of tissue factor in endothelial cells and monocytes is partly regulated by the proinflammatory cytokines tumor necrosis factor and interleukin-1. Also, interaction of tissue factor and P-selectin accelerates the rate and extent of fibrin formation [10].

In the presence of cardiovascular risk factors, not all individuals respond with an arterial thrombotic process, suggesting that several additional factors might be involved in coronary events; for instance, behavior of plasmatic coagulation activation, local blood flow conditions (shear stress), circulating progenitor cells, and genetic factors. Inappropriate generation of thrombin may lead to vascular occlusion [11]. In the atheroma environment, procoagulant and anti-coagulant forces together with pro / anti fibrinolytic substances determine a delicate balance.

Hereditary or acquired defects of blood clotting factors, impairment of the anticoagulant system or fibrinolytic

mechanism, and inflammation, could promote plasmatic and local hypercoagulation state. Local thrombin generation not only results in a mixed, fibrin/platelets clot but thrombin itself has proinflammatory activity [12]. Plaque in unstable angina possesses elevated levels of tissue factor that could be released during inflammation, precipitating acute clinical syndromes.

Local hemodynamic forces play an important role in thrombogenesis in at least two ways: first, physically, as modulators of endothelial function [13,14] by decreasing the vasodilator substances prostacyclin and nitric oxide, or by increasing the vasocontrictor endothelin-1; second, as in situ modulators of clotting balance (triggering platelet activation and over-expression of tissue factor), of fibrinolysis, and of thrombin generation.

The role of inflammation in thrombosis probably varies according to the pathogenesis of the syndrome. With the focus on coronary occlusive disease, there are other origins of thrombotic events beside plaque rupture [15,16]. In atheroma the luminal surface is irregular and sometimes eroded, and the lack of endothelial cells constitutes a vulnerable site, as prone to acute thrombosis as lipid-rich plaques are [17]. The flow zone distal to the apex of the plaque, characterized as a "low shear-stress" region, is prone to fibrinogen deposition [18] and involves areas of flow recirculation, stagnation points and flow reversal, with changes in the metabolic activities of endothelial cells [19]. Blood turbulence produces platelet and clotting activation, accelerates thrombin formation and promotes a mixed thrombus [14]. In these circumstances, platelets are not only involved in haemostasis but also in initiating the inflammatory response.

CD40 ligand (CD40L, CD154), a transmembrane protein structurally related to the cytokine TNF-alpha, is of paramount importance in the development and function of the humoral immune system. Activated platelets express CD40L and induce endothelial cells to secrete chemokines and to express adhesion molecules, indicating that platelets could initiate an inflammatory response of the vessel wall [20].

According to a recent report, bone marrow cells give rise to most of the smooth muscle cells that contribute to plaque formation [21]. Circulating endothelial progenitor cells contribute to the repair capacity of endothelium [22]. Whether endothelial injury in the absence of sufficient circulating progenitor cells affects the balance between injury and the repair capacity of endothelium, determining the progression of the lesion and of cardiovascular disease, is a matter of debate [22] that may provide important insights into the links between inflammation and atherosclerosis.

Genetic predisposition to atherosclerosis was studied in the general population, using carotid artery intima-media thickness as a measure. A positive parental history of myocardial infarction or stroke was associated with increased carotid artery thickness at specific sites in the carotid tree, independently of conventional risk factors [23,24]. Follow-up studies of coronary stenting provided additional support for the central role of inflammation. Indeed, stent produces a prolonged, intense inflammatory state with recruitment of leukocytes, mainly monocytes. There is strong link between the extent of medial damage, inflammation and restenosis [25]. Additional data were reported by Moreno et al. [26]. These authors found a positive correlation between the number of macrophages present in the tissue at the time of angioplasty and the propensity for restenosis.

Markers of Inflammation

As result of chronic inflammation, numerous markers such as CPR (C-reactive protein), cytokines (interleukin-6 and 18, tumor necrosis factor α), adhesion molecules (ICAM-1), E-selectin and acute-phase reactants related to the clotting system (e.g. fibrinogen) are increased in plasma, possible predictors of further cardiovascular events [27–32]. Interleukin-18 plays a key role in the inflammation cascade and is an important regulator of both innate and acquired immunities [33]. It induces the production of interferon-γ and T-lymphocytes, has been found in human atherosclerotic lesions, and was identified as a strong independent predictor of death from cardiovascular causes in patients with stable as well as unstable angina. Inhibition of interleukin-18 reduced lesion progression with a decrease of inflammatory cells.

Matrix metalloproteinase (MMP-9) (gelatinase B), secreted by macrophages and other inflammatory cells, has been identified in various pathological processes such as general inflammation, tumor metastasis, respiratory diseases, myocardial injury, vascular aneurysms, and remodeling. MMP-9 is elevated in patients with unstable angina [34]. Blankenberg et al. [35] noted a strong association between baseline MMP-9 levels and future risk of CV death, independent of IL-18. Combined determination of MMP-9 and IL-18 identifies patients at very high risk.

Proinflammatory cytokines derived from monocytes, macrophages and/or adipose tissue trigger CRP in the liver. C-Reactive protein is an acute-phase reactant, a marker of inflammation, and predicts early and late mortality in patients with acute coronary syndromes. It is an independent predictor of future cardiovascular events [36]. CRP itself promotes inflammation [37] and atherogenesis via effects on monocytes and endothelial cells and increasing the concentration and activity of plasminogen

activator inhibitor-1 [38]. CRP in atheroma participates in the pathogenesis of unstable angina and restenosis after coronary intervention [39]. Thus, there is a vicious circle: inflammation releases proinflammatory cytokines, which in turn maintain inflammation. In a subset of healthy men in the Physicians Health study, the benefit of aspirin (325 mg/day every other day) was most significant in patients within the highest quartile of C-reactive protein elevation compared with the lowest quartile. In patients with coronary artery disease, aspirin also seems to reduce C-reactive protein levels [40]. Components of the metabolic syndrome (obesity, hypertension, hypertriglyceridemia, low HDL, abnormal glucose) are associated with increased levels of CRP and add prognostic information on further cardiovascular events [41]. Statin as aspirin therapy was particularly effective among patients with high CRP levels [30]. It seems clear that CRP is a marker for risk for cardiovascular events, but whether it should be used in routine screening is still a matter of debate [42–45].

Inflammation Mediate Risk Factors

Pleiotropic atheroprotective activity of specific treatments involving antiinflammatory properties

Diabetes

Although diabetes mellitus is primarily a metabolic disorder, it is also a vascular disease [46].

The most important cause of death among diabetic patients is cardiovascular complication. Comparing diabetic patients with non-diabetic patients both with or without prior history of cardiovascular events, Haffner et al. [47] showed, after a follow-up of 7 years, that mortality in diabetic patients was higher than in non-diabetics and that for diabetic patients with no history of myocardial infarction, the risk of myocardial infarction was similar to that of non-diabetic patients who did have such a history. These data suggest that diabetic patients have already developed vascular disease by the time of clinical diagnosis [46]. Although type 2 diabetes is a state of increased plasma coagulability [48], it is clear that endothelial dysfunction is the most important factor in thrombotic complications. It is present mainly in type 2 diabetes than type 1 [49,50]. The so called metabolic syndrome, a concurrence of disturbed glucose and insulin metabolism, overweight and abdominal fat distribution, moderate dyslipemia and hypertension, might be responsible for the vascular endothelial dysfunction. Indeed, cardiovascular disease and all causes of mortality are increased in men with metabolic syndrome [51].

As indicators that the endothelium is compormised, microalbuminuria and proteinuria are frequently present in diabetic patients and constitute predictors of cardiovascular events and ischemic stroke [52–54]. Hyperglycemia and production of advanced glycation end products (AGE) are probably the most important factors, if not the only factors, in endothelial dysfunction in diabetics. By binding specific receptors (RAGE), AGE induces the expression of different proinflammatory molecules. Endothelial dysfunction could be also consequence of the dyslipidemia frequently present in type 2 diabetes and oxidative stress [55].

Diabetic patients have impaired endothelium-dependent vasodilatation, hyper-coagulability, increased PAI-1 level in the arterial wall with impaired fibrinolysis, decrease of endothelial nitric oxide synthase, and increase of endothelin-1 [56]. Platelets are larger, with an increased number of glycoprotein IIb-IIIa receptors in the membrane, and are hyper-reactive and show enhanced biosynthesis of thromboxane A2 [57,58]. Platelets from diabetic subjects behave abnormally, showing increased adhesiveness as well as spontaneous and agonist-induced aggregation, reflected by abnormalities in platelet function tests [59,60]. Von Willebrand factor, mainly synthesized by endothelial cells and involved in platelet adhesion, is increased, reflecting endothelial activation or damage [61].

Although diabetic patients presented with thrombophilic profiles, in diabetics with acute coronary syndromes without ST-segment elevation, or following percutaneous coronary intervention, inhibitors of the platelet receptors glycoprotein IIb-IIIa reduced mortality compared with non-diabetics. This remains unexplained at present and is at odds with the hyper-reactivity of platelets seen in these patients [62,63].

It is known that cytokine release and processes leading to macrophage activation are enhanced in diabetics [64] and contribute to the development of athero-inflammatory complexes. The blood level of C-RP is generally higher in diabetic patients than in normal populations indicating that inflammation contributes to the development of the disease [65]. Low grade inflammation is involved in the pathogenesis of type-2 diabetes. Results from the MONICA study [66] showed that men with CRP in the highest quartile (≥ 2.91 mg/L) had a 2.7 times higher risk of developing diabetes than those in the lower quartile (≤ 0.67 mg/L).

Lastly, a novel group of antidiabetic drugs, thiazolidinediones, exhibit anti-inflammatory properties in addition to their plasma glucose lowering effect. Type 2 diabetic patients with coronary artery disease have high levels of inflammatory markers, including serum C-reactive protein, metalloproteinase-9, white cells count, tumor necrosis factor-α and serum amyloid A [67,68]. Since treatment with rosiglitazone significantly reduces these

inflammatory markers it could indicate a link between diabetes, coronary disease and inflammation.

Lipids

In atherosclerosis, signs of inflammation are accompanied by incipient lipid accumulation in the artery wall [69]. Several factors determine endothelial modifications through a primary inflammatory response followed by a local prothrombotic balance.

LDL oxidation is a main cause of endothelial injury and induces the expression of proinflammatory molecules in endothelial cells. Thus removal of modified LDL is important in the treatment of the inflammatory response.

Several studies have reported high levels of total tissue factor pathway inhibitor (TFPI) antigen in patients with serum elevated cholesterol. Increased TFPI inhibit the extrinsic coagulation system, but the procoagulant system may be activated concurrently. The median levels of total TFPI, free TFPI, FVIIc and prothrombin fragments 1+2 were higher in hyperlipidemic patients than healthy subjects. The increase of fragments 1+2 indicates a global thrombophilic state [70,71]. These blood changes follow the endothelial inflammatory reaction.

Other lipids have also active inflammatory effects; notably, high plasma levels of VLDL are associated with increased risk of atherosclerosis. In this regard, Dichtl et al [72] showed that VLDL (75 to 150 μg/mL) activates nuclear factor-κB (NF-κB), a transcription factor known to play a key role in regulation of inflammation, in cultured human endothelial cells. There was also expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, as well as proinflammatory molecules such as the cytokine tumor necrosis factor-α. Injected triglycerides, precursors of LDL, also activate arterial expression of NF-κB. In line with this result, postprandial hypertriglyceridemia is considered a risk factor for cardiovascular disease. It was suggested that postprandial hypertriglyceridemia induces endothelial dysfunction through oxidative stress [73].

Lipoprotein (a) has a structure similar to that of plaminogen and may reduce plasmin generation and impair fibrinolysis, inducing a prothrombotic state. Elevated levels of lipoprotein (a) might strongly predict endothelial dysfuction in normocholesterolemic and non-diabetic subjects [74].

Oxidative inactivation of nitric oxide is regarded as an important cause of endothelial lesion. Decreased nitric oxide may favor platelet-adhesion/aggregation and arterial thrombosis. One of the pathophysiological consequences of platelet binding to LOX-1 may be the inactivation of nitric oxide (NO) through increased cellular production of O_2 [75].

Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) reduce cardiovascular disease events and improve outcomes. These benefits have been attributed to their LDL-lowering (and potentially HDL-elevating) effects. However, several trials have suggested that the clinical benefit of statins is greater than expected from the lowering effect on lipids [76]. Analysis of large clinical trials indicates that statin-treated individuals have significantly less cardiovascular disease irrespective of their serum cholesterol levels and the treatment is particularly effective among patients with high CRP levels [77,78]. Long-term statin therapy has been shown to reduce levels of C-reactive protein in a lipid-independent manner [79]. Additional effects were observed: statins limit tissue factor expression [80], normalize fibrinolytic activity, reduce plaque inflammation and cause regression of human atheroesclerotic lesions [81], improve coronary endothelial function [82] and have antiinflammatory and antiproliferative effects [83]. In this regard, statins are potent upregulators of endothelial cell nitric oxide synthase levels and nitric oxide synthesis. In a rabbit model and in cultured vascular smooth muscle cells, atorvastatin decreased inflammatory mediators in the atherosclerotic lesion and significantly downregulated COX-2 both in vitro and in vivo [84]. Other effects may also be mediated through nonlipid changes: Statins significantly increase circulating endothelial progenitor cells [85], contributing to the repair capacity of endothelium [21]. Also, statins exert beneficial effects in vascular diseases by inhibition of leukocyte rolling, adherence, and transmigration. All statins are potent reversible inhibitors of the key enzyme in cholesterol synthesis but their pharmacological profiles differ [86]. For instance, it was found that atorvastatin reduced CPR and serum amyloid whilst simvastatin had little or no effect on these variables. Levels of IL-6 and ICAM-1 were also inconsistent and little modified [87].

Moderate drinking had a lipid lowering effect and alcohol intake, at least three to four days per week, was associated with protection from cardiovascular events. The risk was similar among men who consumed less than 10 g of alcohol per drinking day and those who consumed 30 g or more. No single type of beverage conferred additional benefit [88]. Alcohol intake also lowered the C reactive protein level, independently of effects on lipids, indicating antiinflammatory activity [89]. Thus, there are several effects of statins besides their lipid lowering activity that could be attributed to their antiinflammatory capacity and could be relevant to the improvement of altered local and systemic factors.

Obesity

It has been consistently reported that increasing degrees of obesity are accompanied by greater rates of cardiovascular disease [90,91]. Obesity is an independent risk factor for major coronary events although hypercholesterolemia and the metabolic syndrome are often associated with it [92–94]. Obese subjects typically carry a proinflammatory state that may predispose them to acute coronary syndromes. This state is characterized by elevations of serum CRP that reflect high cytokine levels [95]. Excess adipose tissue secretes increased amounts of several inflammatory cytokines. Interestingly, weight loss produces a reduction of CRP levels [96], and serum concentrations of IL-6, IL-18 and adiponectin levels are increased significantly [97]. This indicates that weight control could diminish the inflammatory state. Adipose tissue present in excess also releases increased amounts of plasminogen activator inhibitor-1 (PAI-1), which imbalances the fibrinolytic system towards prothrombosis [98,99].

Leptin, a circulating hormone produced by adipose tissue, regulates body weight and food intake and metabolism. It can influence vessel tone and an increase amount of leptin, as in obesity, could contribute to arterial vessel stiffness, impaired vascular function and cardiovascular events. Leptin has angiogenic activity, increases oxidative stress in endothelial cells which could contribute to vascular pathology, and promotes vascular cell calcification and smooth muscle cell proliferation and migration [100,101]. Although the release of leptin may cause local vasodilation mediated by nitric oxide, with time it increases oxidative stress, followed by a decrease of bioactivity and / or synthesis of nitric oxide and increase of inflammatory mediators [92].

Inflammation as an immune-mediated disease. Role of infection

Immunity

Mediators of innate and acquired immunity are involved at various stages of atherosclerosis, as might be anticipated for a chronic inflammatory process [102]. In the chronic state, atheromata contain immune cells: T lymphocytes, activated macrophages and mast cells, which are also present in inflammatory infiltrates. This led to the notion that the inflammatory response is immune-mediated, and the involvement of immune mechanisms in atherosclerosis was postulated [103]. Innate immune reactions against bacteria and viruses have been included in the list of pathogenic factors in atherosclerosis. Toll Like Receptors (TLR), known to play a key role in the innate immune response, are expressed in atherosclerotic plaques and are associated with inflammatory activation of endothelial cells and macrophages. The family of toll like receptors, mainly TLR-1, 2 and 4, expressed at low levels in normal endothelium, are markedly increased in macrophages and endothelial cells of human atherosclerotic lesions. Expression of TLR in cultured vascular endothelial cells was increased by stimulation with proinflammatory cytokine [104]. Lipopolysaccharides released during acute infection might link the immune response, bacterial infection and inflammation through TLR activation in plaque cells, endothelial cells and macrophages. This suggests a mechanism by which microbes may cause inflammatory plaque activation. *Chlamydia pneumoniae* may signal through TLR to induce the proliferation of human vascular smooth muscle cells [105].

A recent paper by Kiechl et al [106] offers an additional and interesting contribution to the potential importance of TLR in the relationship between the inflammatory response to gram-negative pathogens, innate immunity and atherogenesis. These authors found that patients with TLR4 polymorphism have lower levels of proinflammatory cytokines, acute phase reactants, and soluble adhesion molecules. Such subjects are more susceptible to severe bacterial infection, but they have lower risk of atherosclerosis as assessed by high-resolution duplex ultrasonography of the carotid artery.

Some research has suggested that acute respiratory infection may be a risk factor for myocardial infarction. An increase of acute coronary events during winter infections and flu epidemics has been related to seasonal variations in factor VIIa and fibrinogen, probably induced via activation of the acute phase response [107,108]. In these circumstances an immune response could support an inflammatory process and might be associated with increased trafficking of macrophages into the artery wall [109]. Some studies support the assumption that influenza vaccine reduces the risk of recurrent MI in patients with documented coronary heart disease. Naghavi et al [110] provided indications that in patients with chronic coronary heart disease, vaccination against influenza was negatively associated with the development of new myocardial infarction during the same influenza season. The beneficial effect of vaccination against influenza in the elderly was indicated in the recent paper of Nichol et al. [111].

Vaccination was associated with a reduction of 19% in the risk of hospitalization for cardiac disease and cerebrovascular disease (reduction of 16 and 23 percent during the 1998–1999 and the 1999–2000 season respectively). Gurfinkel et al. [112] evaluated the preventive effect of vaccination on ischemic events in myocardial infarction patients and in subjects undergoing planned percutaneous coronary angioplasty. In a small number of patients suffering from infarction, but not in those recovering from angioplasty, influenza vaccination reduced the risk of death and ischemic events.

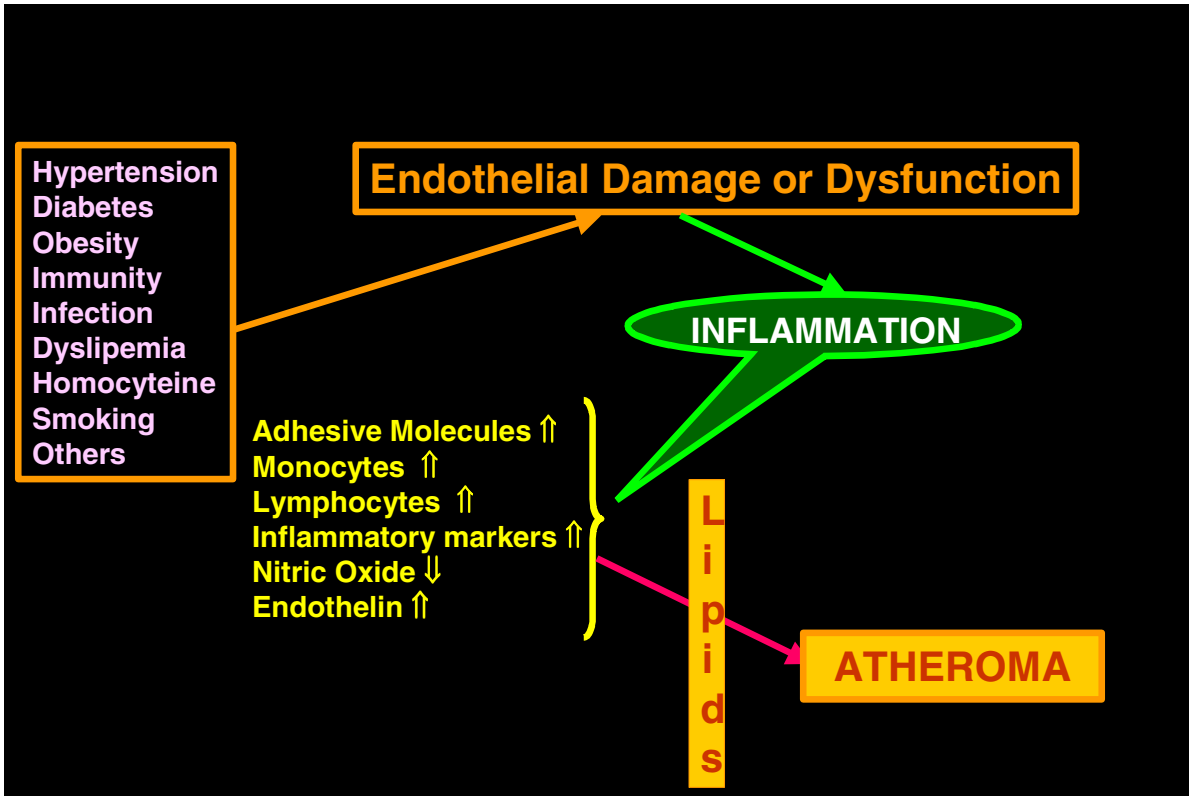

Figure 1

The antiphospholipid syndrome considered an autoimmune disease triggering endothelial cellular disturbance, produces arterial and venous thrombosis [113,114]. The high affinity of antiphospholipid antibody-β_2GPI complex for phospholipid membranes seems to be a critical factor in this disease [115]. Activation of endothelial cells and enhanced thrombosis by antiphospholipid seem to be mediated by ICAM-1, P-selectin, or VCAM-1 [116].

The humoral immune response could be a high risk factor for coronary heart disease, inducing inflammation that links immunity with coronary disease.

Infection

Infection and the immune local reaction cause endothelial dysfuction, cell injury, and a proinflammatory environment. Whether infection is the main factor involved in inflammation remains unproved [117–125]. Endotoxins secreted by bacteria are potent activators of different inflammatory reactions, stimulating circulating monocytes and causing production of several cytokines. They may also disturb hemostasis [126].

Interleukin-18 gene expression is stimulated by proinflammatory cytokines and also by lipopolysaccharides. If infection is also a trigger of interleukin-18 this could explain the relationships between inflammation and infection and the controversial association between previous infection and cardiovascular events. It could also explain the erratic effects of antibiotics on the prevention of coronary artery disease.

Expression of toll like receptors in atheroma is a suggested mechanism for inflammatory plaque activation by microbes [105].

Many seroepidemiology studies suggest a relationship between infection and the pathophysiology of ischemic heart disease and the severity of atherosclerosis [126,127]. *Chlamydia pneumoniae*, cytomegalovirus and Helicobacter pylori have been associated with atherosclerotic lesions. Moreover, viral and bacterial proteins can induce antiphospholipid antibody production in humans which could be an additional factor attacking endothelium [128]. Of these candidate organisms, *Chlamydia pneumoniae* appears most likely to be involved in coronary disease through different mechanisms. *Chlamydia pneumoniae* can be replicated and maintained in human macrophages and in endothelial cells. Thus it can participate in the acute coronary process through a direct effect on atheroma, initiating the inflammatory process, or it can remain latent in the atheroma as a bystander, subsequently being activated during inflammation and acutely exacerbating the response. Alternatively, atheroma might be colonized by *Chlamydia pneumoniae* during plaque inflammation, contributing to plaque disruption.

The controversial role of *Chlamydia pneumoniae* in coronary events was also indicated by the effect of antibiotic treatment. *Chlamydia pneumoniae* is sensitive to macrolides (azithromycin, roxithromycin and clarithromycin) [129], but besides their anti-infectious activity, an alternative mechanism for macrolides was suggested [130–132]: they could suppress macrophage activity, which means they could have antiinflammatory effects, different for each drug. Controversial results could be related to these different antiinflammatory effects.

Homocysteine

Elevated circulating homocysteine (tHcy) level is a risk factor for occlusive disease in the coronary, cerebral, and peripheral vessels and predictive of survival in patients with stable coronary artery disease. Nevertheless a causal relation still remains to be proven [133–136].

The Homocysteine Studies Collaboration concludes that evidence of a link between higher homocysteine levels and the risk of coronary disease is weaker than previously reported [137]. It had been suggested that serum homocysteine level on hospital admission was an independent predictor of long-term survival in patients with acute coronary syndromes, but a meta-analysis of the observational studies suggests only a modest independent prediction of ischemic heart disease and stroke risk in healthy populations. Nurk et al. [138] provide evidence that plasma homocysteine level is a strong predictor of cardiovascular disease only in elderly patients, and especially among those with preexisting cardiovascular disease. Thus homocysteine interacts with conventional risk factors to provoke the acute event. Klerk et al. [139] indicate that individuals with the MTHFR 677 TT genotype have a significantly higher risk of coronary heart disease, particularly in the setting of low folate status, and support the hypothesis that impaired folate metabolism, resulting in high homocysteine levels, is causally related to a 16% increased risk of coronary heart disease. Nevertheless, the excess risk was evident only in European studies, not in North American investigations [139].

The link between homocysteine and coronary disease may be mediated by activation of coagulation and alteration of the vasomotor regulatory and anticoagulant properties of endothelial cells [140–142]. Impaired homocysteine metabolism may result in oxidative stress [143], which might play a central role in hyperhomocysteinemia-mediated vascular disorders [144,145]. Homocysteine increases TNF-expression, which enhances oxidative stress and induces a proinflammatory vascular state that may contribute to the development of coronary atherosclerosis [146].

It has also been suggested that enhanced peroxidation of arachidonic acid to form bioactive F_2-isoprostanes could represent the mechanism linking hyperhomocysteinemia to platelet activation in cystathionine β-synthase deficient patients [147].

A recent report showed that folic acid, vitamin B_{12}, and pyridoxine significantly reduce homocysteine levels, the rate of restenosis and the need for revascularization after coronary angioplasty [148,149]. But in the paper by Doshi et al., folic acid 5 mg/d for 6 weeks improved endothelial function, as assessed by flow-mediated dilatation in cardiovascular artery disease, by a mechanism independent of homocysteine [150].

Hypertension

The renin-angiotensin system contributes to the pathogenesis of atherosclerosis. Its effect on blood pressure partially explains this; also, angiotensin II may elicit inflammatory signals in vascular smooth muscle cells. The transcription factor NF-κB participates in most signaling pathways involved in inflammation [151]. Angiotensin II is a regulator of the NF-κB family and may be responsible for activating the expression of cytokine gene networks in vascular smooth muscle cells. It can also promote long-term changes in vascular smooth muscle cell function by its ability to induce cellular hypertrophy, extracellular matrix production, and early gene expression [152]. Angiotensin II also activates inflammatory pathways in human monocytes.

L-Arginine, a nitric oxide precursor that augments endothelium-dependent vasodilatation, acutely improves endothelium-dependent, flow-mediated dilatation of the brachial artery in patients with essential hypertension

[153]. As mentioned during the discussion of other risk factors, inflammation may link hypertension and atherosclerosis, and the clinical benefits of treatment with angiotensin-converting enzyme inhibitor may to some extent derive from interrupting inflammation [31].

Smoking

Cigarette smoking is a major risk factor for developing coronary artery disease, producing a marked decline in endothelium-dependent vasomotor response [154–156]. It causes endothelial dysfunction, possibly through increased oxidative stress, and this is also true for passive smoking or environmental tobacco smoke. A 30-minute passive smoking exposure was found to affect coronary flow velocity reserve in nonsmokers [157]. Light and heavy smoking have similar detrimental effects on endothelium-dependent vasodilation and the nitric oxide biosynthetic pathway [158]. Significant increases of sICAM-1 and sVCAM-1 were demonstrated in smokers, and nitric oxide metabolites were reduced significantly [159]. Smoking-induced endothelial dysfunction of resistance vessels is rapidly reversed with oral allopurinol. These data suggest that xanthine oxidase contributes importantly to the endothelial dysfunction caused by cigarette smoking [160]. Folic acid significantly improves endothelial function in otherwise healthy cigarette smokers. This provides a potential therapeutic tool for attenuating the atheromatous process in this group [161].

Conclusions

The new findings add evidence for a close relationship between risk factors, inflammation and atherosclerosis. Inflammation is the common response of endothelial cells to different factors that attack arterial intima. Taking into account this chain of local arterial endothelial cell reactions, the behavior of inflammation markers, and the effects of specific drugs that possess additional anti-inflammatory effects, the concept of athero-inflammation emerges as the meeting point of different morbidities, usually named as risk factors, which include dyslipemia, diabetes, hypertension, obesity, immunity, infection, hyperhomocyteinemia, smoking (Figure).

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