REVIEW

Inflammatory and thrombotic mechanisms in coronary atherosclerosis

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Many molecular and cellular mechanisms link inflammation and haemostatic mechanisms. Inflammation, and perhaps chronic infection, may play important roles in the initiation and progression of atherosclerosis. Atherosclerotic lesions are heavily infiltrated by cellular components associated with inflammation (macrophages and T lymphocytes), and acute plaque rupture is also associated with inflammatory components. Several markers of systemic inflammation may predict future cardiovascular events in apparently healthy subjects as well as in patients with chronic and acute syndromes. There may thus be therapeutic potential in modifying the atherosclerotic, vasomotor, and thrombotic components of ischaemic heart disease.

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Coronary thrombosis is now widely recognised as a major cause of sudden cardiac death, acute myocardial infarction, and unstable angina pectoris. Inflammation is an important component of the atherosclerotic lesion. In this review we will discuss inflammatory mechanisms in relation to atherosclerosis and clinical coronary thrombosis.

The "response to injury" hypothesis postulates that endothelial dysfunction represents the initial step of atherogenesis and can be induced by haemodynamic forces, by a variety of vasoactive substances, by mediators from blood cells, and directly from risk factors for atherosclerosis.[1] Upon activation, endothelial cells express various cellular adhesion molecules, cytokines, chemokines, and growth factors. Focal arterial inflammatory activity is one of the most prominent characteristics of the atherosclerotic process.[2 3] Inflammation is also implicated in the pathogenesis of acute syndromes, as suggested by histological findings in unstable coronary plaques,[4–6] evidence of systemic release of thromboxanes and leukotrienes,[7–9] and the presence of activated circulating leucocytes.[10 11] A process involving predominantly mononuclear leucocytes followed by fibrosis and finally tissue degeneration is common to many inflammatory disorders apart from atherosclerosis.

High sensitivity testing for C reactive protein (CRP)—a non-specific plasma marker of low grade systemic inflammation—has received much attention, and the results of several studies show a strong link between baseline elevations of CRP and the risk of future cardiac events.[12–15] Other acute phase proteins also have prognostic significance in coronary artery disease.[16 17] The leucocyte count and various inflammatory proteins such as fibrinogen,[18 19] plasminogen activator inhibitor (PAI-1),[16] von Willebrand factor,[20] albumin,[18] and various cytokines and adhesion molecules have been found to be independently associated with cardiovascular end points.[21 22] Increased concentrations of interleukin 6 (IL-6), the major cytokine responsible for the acute phase response, are common in unstable patients and also correlate closely with prognosis.[23] It has been suggested that the association between *Chlamydia pneumoniae* and clinical events[24–27] may be because chlamydiae invade macrophages and exacerbate the inflammatory process within the plaque.[28]

CELLULAR AND MOLECULAR MECHANISMS INVOLVED IN INFLAMMATION

B and T lymphocyte in atherosclerosis

T cell adhesion to dysfunctional endothelium has been demonstrated in vivo and in vitro.[29 30] T cells of both the helper (CD4+) and cytotoxic/suppressor types have been detected in human atheroma and have been shown to be immunologically activated.[31] The first direct evidence for this activation was the demonstration of class II histocompatibility antigen expression on the surface of smooth muscle cells adjacent to the T lymphocytes in the lesions. This human lymphocyte antigen (HLA) expression is induced by interferon-γ, a product of activated T cells and natural killer cells. As natural killer cells are not found in complicated plaques, the only remaining source of interferon-γ is the adjacent activated T cells. The presence of activated T lymphocytes in the atherosclerotic plaque suggests a local immune response, and it has been postulated that such a response may be directed against local antigens in the plaque.[32 33] Activated T lymphocytes secrete growth factors and cytokines that may affect other cell types and the process of atherosclerosis.

Abbreviations: CRP, C reactive protein; ICAM, intercellular adhesion molecule; IL, interleukin; LDL, low density lipoprotein; Lp(a), lipoprotein(a); MCP, monocyte chemoattractant protein; NF-κB, nuclear transcription factor κB; PAI, plasminogen activator inhibitor; TGF, transforming growth factor; TNF, tumour necrosis factor; VCAM, vascular cell adhesion molecule; VLA, very late activation antigen

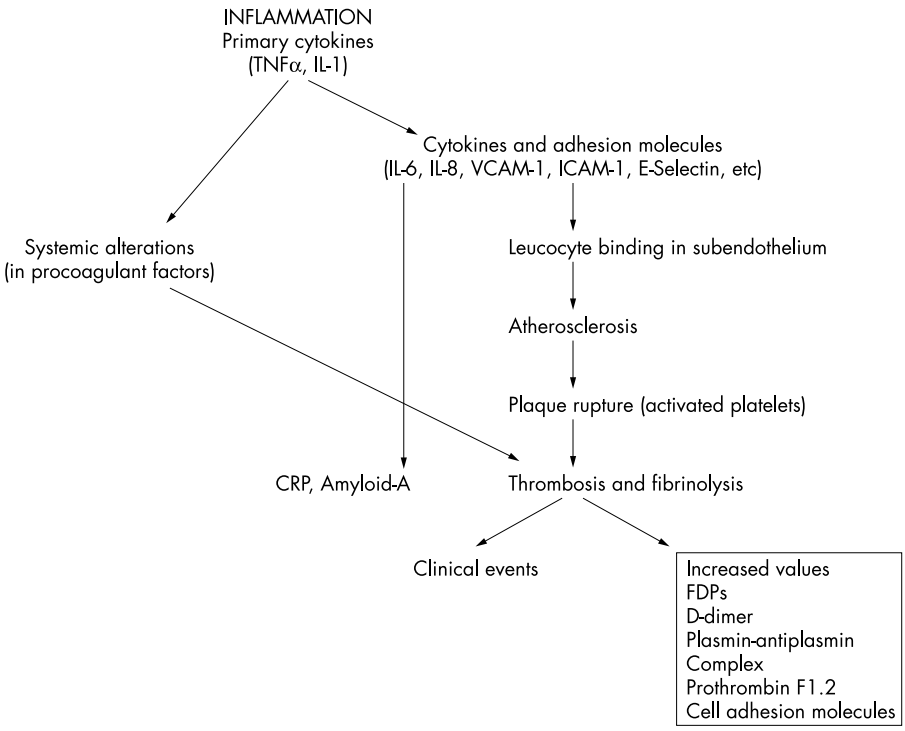

Figure 1 Links between inflammation and thrombotic mechanisms in atherosclerosis. Inflammation acting both locally and systemically may initiate atherosclerosis and promote thrombosis by weakening the fibrous cap of the atheromatous plaque leading to plaque rupture.

Monocytes and macrophages in atherosclerosis

Arterial leucocyte recruitment in early atherosclerosis also involves monocytes.[34] Following endothelial adhesion and transmigration into the arterial intima, these cells express markers of activation such as HDL-DR, IL-2 receptor, and very late activation (VLA) antigen. Interleukins, complement factor fragments, and tumour necrosis factors (TNF) can enhance monocyte adhesiveness and chemotaxis and so form an amplification mechanism for recruitment of further monocytes into the lesion.[35] Furthermore, released mitogens, such as macrophage derived growth factor, may play a key role in smooth muscle cell migration and subsequent proliferation.[36]

Activation of circulating leucocytes may be facilitated at the endothelium covering an atherosclerotic plaque, with upregulation of adhesion molecules and tethering of circulating cells. These inflammatory responses, possibly mediated by sCD40L, may further promote the infiltration of activated leucocytes into the atherosclerotic lesion, which in turn may directly activate smooth muscle cells, macrophages, and T cells inside the vessel wall.[37 38] Lesional macrophages produce proteolytic enzymes which include members of the metalloproteinase family.[3] Experimental studies[39–41] have described constitutive expression of metalloproteinases by the macrophage foam cells within atheroma of hypercholesterolaemic rabbits. This macrophage related proteolysis within the atheroma contributes to weakness of the protective fibrous cap of the plaque and hence promotes the propensity of those plaques to rupture and trigger thrombosis. The interaction of macrophages with lymphocytes using CD40 and its ligand also upregulates metalloproteinases.[37]

Cytokines

Cytokines such as tumour necrosis factor α (TNFα) or IL-1 isoforms can stimulate the expression of IL-6, IL-8, and leucocyte adhesion molecules such as intercellular adhesion molecule (ICAM-1). These cytokines are produced by neutrophils and macrophages which are located in atheromatous plaques. They may be derived from non-vascular sources and reflect generalised inflammatory states, such as chronic infection, which have been linked to atherogenesis and its clinical manifestations.[24] The contribution of vascular and extravascular sources of inflammatory cytokines may vary between individuals.

Primary cytokines (TNFα, IL-1) stimulate the production by endothelial and other cells of adhesion molecules, procoagulants, and other mediators that may be released in soluble form into circulating blood (fig 1). Primary cytokines also stimulate the production of messenger cytokine, IL-6, which induces expression of hepatic genes[42] encoding acute phase reactants found in the blood, including CRP and serum amyloid A. In animal and human models of atherosclerosis, the first sign of disease activity is an upregulation of adhesion molecules such as the vascular cell adhesion molecule (VCAM-1), E-selectin, and ICAM.[43] These molecules are instrumental in endothelial leucocyte binding and recruitment of leucocytes into the subendothelial tissue which are essential steps in the initial development of atherosclerotic lesion (fig 1). TNFα also stimulates the expression of IL-6.

In patients with acute coronary syndromes, leucocyte–platelet adhesion is increased in circulating blood, suggesting an enhanced inflammatory response in patients with preinfarction angina.[44] Binding to activated platelets induces IL-1β, IL-8, and monocyte chemoattractant protein 1 (MCP-1) in the leucocytes. These findings suggest that leucocyte–platelet adhesion may contribute to the regulation of inflammatory response in acute syndromes.

LIPOPROTEINS AND INFLAMMATION

Raised concentrations of low density lipoprotein (LDL) and possibly lipoprotein(a) (Lp(a)) may attract monocytes to adhere to endothelium and induce their transformation into macrophages.[45] The proinflammatory effects of oxidised LDL involve peroxides and other reactive oxygen intermediates generated by the oxidation of LDL. These molecules activate nuclear transcription factor κB (NF-κB),[45] which plays a key role in the orchestration of inflammatory and immune responses by controlling the transcription of the genes encoding several of the adhesion molecules, interleukins, TNFα, class II antigen, and antibodies. NF-κB recognises various activators, among which are the proinflammatory cytokines

Table 1 Effects of inflammation on thrombosis–haemostasis

1	Inflammatory cytokines modulate the haemostatic properties of the endothelium
2	Local effects of inflammatory cells on digestion of the fibrous cap lead to plaque disruption and thrombus formation
3	Inflammation can affect systemic haemostatic activity by IL-6 mediated stimulation of hepatocytes to produce acute phase reactants (coagulation factors, PAI-1)
4	Enhanced CD40L-CD40 interaction promotes thrombotic activity by enhancing tissue factor expression in macrophages and through the direct regulation of endothelial procoagulant activity
5	Activated platelets may mediate the homing of leucocytes by interaction with the subendothelial matrix under shear stresses
6	Oxidised LDL induces tissue factor expression in macrophages and decreases the anticoagulant activity of endothelium by interfering with thrombomodulin expression and inactivating tissue factor pathway inhibitor
7	Acute phase reactants are associated with an increased risk of future cardiovascular events which are mediated by acute thrombosis (for example, C reactive protein, fibrinogen, factor VIII)

IL, interleukin; LDL, low density lipoprotein; PAI, plasminogen activator inhibitor.

and CRP. Lp(a) is more abundant in atherosclerotic lesions from acute coronary syndromes and co-localises with macrophages in atherectomy specimens, suggesting interaction with inflammatory cells and extracellular matrices.[46 47] Lp(a) enhances ICAM-1 expression partly by decreasing active transforming growth factor β.[35]

INFLAMMATION AND THROMBOSIS

Inflammatory cytokines modulate the homeostatic properties of the endothelium.[1] The local effects of inflammatory cells on digestion of the fibrous cap lead to plaque disruption and thrombus formation. If the thrombi are small they may organise and contribute to the growth of the atherosclerotic plaque. If the thrombi are large or occlusive, they lead to the acute coronary syndromes.

Certain thrombogenic risk factors may modulate the degree of thrombogenicity and thereby determine growth of the plaque and the occurrence of the various acute coronary syndromes.[16 48] Tissue factor is normally expressed in exposed intima and activates factor VII which in turn activates factors IX and X. Collagen in exposed intima binds von Willebrand factor, which mediates platelet adherence by binding to the glycoprotein Ib/V/IX platelet surface receptor complex under high shear stress conditions. von Willebrand factor itself is the carrier protein for factor VIII, an essential component of the amplifying mechanism of the factor X–Xa conversion. Furthermore, platelets activated by adhesion adhere to the other platelets through the glycoprotein IIb/IIIa receptor and its ligand, von Willebrand factor and fibrinogen. Such activated platelets release PAI-1, which locally inhibits the fibrinolytic mechanism.[49]

Inflammation may promote thrombosis by acting both locally and systemically. Local mechanisms include the cytokine stimulated expression of tissue factor by endothelial cells and macrophages. Indirectly, inflammation may act locally to induce thrombosis by weakening the fibrous cap of the atheromatous plaque, leading to plaque rupture. However, this role of inflammation, and specifically the role of macrophages, remains controversial.[50]

Inflammation can affect systemic haemostatic activity by IL-6 mediated stimulation of hepatocytes to produce acute phase reactants. These include certain coagulation factors, such as increased levels of fibrinogen and PAI-1, which induce a prothrombotic state (table 1).

An enhanced CD40L–CD40 interaction also promotes thrombotic activity by enhancing tissue factor expression in macrophages and through the direct regulation of endothelium procoagulant activity.[38 51] Intravascular fibrinolysis induced by tissue type plasminogen activator may contribute to atherosclerosis by inducing P-selectin and platelet activating factor, as well as to plaque rupture by activating metalloproteinases[52] (table 1).

Table 2 Platelets and inflammatory responses

1	Activated platelets may mediate the homing of leucocytes by interaction with the subendothelial matrix under shear stresses that do not allow neutrophil adhesion
2	Activated platelets contribute to the oxidative modification of LDL
3	Activated platelets contribute to smooth muscle cell proliferation
4	Activated platelets contribute to inflammatory reactions by expressing and releasing CD40L, resulting in MMP activation and procoagulant activity

LDL, low density lipoprotein; MMP, matrix metalloproteinase.

Oxidised LDL also induces tissue factor expression in macrophages and decreases the anticoagulant activity of endothelium by interfering with thrombomodulin expression and inactivating tissue factor pathway inhibitor.[53] Its expression is upregulated in circulating and endothelium adherent monocytes, and tissue factor has been found to be increased in coronary tissue of the culprit lesion from patients with unstable angina[54–56] (table 1).

Recently, several studies have indicated that raised concentrations of CRP are associated with increased risks of future cardiovascular events. Initially, Liuzzo and colleagues and Haverkate and associates established the prognostic value of CRP in the setting of stable and unstable angina.[14 57] Other acute phase reactants have also been used to indicate increased risk of cardiovascular events.[58] Both fibrinogen[59 60] and increased factor VII and VIII concentrations[61 62] have been found to predict cardiovascular events in an independent manner. PAI-1 predicts second myocardial infarction in survivors of a first infarct.[48]

It is also now accepted that platelets may promote inflammatory responses. Studies have shown that activated platelets may mediate the homing of leucocytes by interaction with the subendothelial matrix under shear stresses that do not allow neutrophil adhesion.[61 62] They may also contribute to the oxidative modification of LDL, provide a source of lipids for foam cell generation, and contribute to smooth muscle cell proliferation. Platelets from patients with unstable angina are characterised by notably decreased intracellular sCD40L concentrations as well as by decreased release of sCD40L (table 2).[63]

CHRONIC INFECTIONS AND INFLAMMATION

Experimental and clinical studies have suggested that there is a significant association between ischaemic heart disease and various infective diseases, both bacterial and viral, including cytomegalovirus, chronic bronchitis, and dental infections.[64–66] More virulent *Helicobacter pylori* strains bearing the cytotoxin association gene A have a well recognised pathogenic role in peptic ulcer disease and gastric cancer[67] and directly induce enhanced inflammation.[68] A recent study[69] supported the hypothesis that *H pylori* may influence atherogenesis through low grade, persistent inflammatory stimulation. It has been also shown that *Chlamydia pneumoniae*, cytomegalovirus, and *H pylori* may be detected within human atherosclerotic tissues.[70–73] It has been hypothesised that these organisms may activate vessel associated leucocytes or lead to a transformation of vascular smooth muscle or endothelial cells.[24] Another study[74] showed associations of antibodies to *C pneumoniae, H pylori*, and cytomegalovirus with immune reactions to heat shock protein 60 and carotid or femoral atherosclerosis. Small trials[27 75] have demonstrated that macrolide antibiotics which are active agents against *C pneumoniae* might reduce the

cardiovascular event rate. Large studies are going on to explore the possible relation between antibiotics and clinical coronary events.

CLINICAL RELEVANCE

It has been suggested that inflammation with subsequent thrombus formation provides a potential explanation for the substantial percentage of patients who suffer an acute coronary event without evidence of any traditional risk factors for atherosclerosis.[16 17 59 60 76 77] Lipid lowering treatment with pravastatin significantly reduces the serum concentration of inflammatory markers even when the lipid concentration has stabilised.[78 79] Lipid lowering by dietary manipulation reduces proteolytic activity of macrophages and increases collagen content of established atheroma in rabbits.[41] Lipid lowering treatment may also increase plaque stability by several other mechanisms, including reducing the size of the lipid core and decreasing inflammation, restoring endothelial function, decreasing tissue factor expression, and reducing the thrombosis.[39–41 80] Abnormal coronary vasomotion and forearm blood flow abnormalities have been found in hypercholesterolaemic patients, and normalisation of function has been achieved by statin treatment.[81–83] Chronic infections may be related to acute coronary syndromes, although their exact role is still to be validated.[75 84] High doses of aspirin also reduce the risk of subsequent cardiovascular disease in apparently healthy men, and reduce their CRP concentrations.[85] In a recent study from our group, thermal heterogeneity within human atherosclerotic coronary arteries was detected in vivo by application of a special thermographic catheter[86]; this correlated with the plasma CRP concentration.

CONCLUSIONS

Increasing numbers of molecular and cellular mechanisms have been identified which link inflammation and haemostatic mechanisms. Inflammation, and perhaps chronic infection, may play important roles in the initiation of atherosclerosis and progression to its final stages. Atherosclerotic lesions are heavily infiltrated by cellular components associated with inflammation (macrophages and T lymphocytes), and acute plaque rupture is also associated with inflammatory components. At present there is little evidence for a role of infections in acute coronary syndromes and thrombosis. Several markers of systemic inflammation may predict future cardiovascular events in apparently healthy subjects, as well as in patients with chronic and acute syndromes. There may therefore be therapeutic potential in modifying the atherosclerotic, vasomotor, and thrombotic components of ischaemic heart disease—directly or indirectly—by using anti-inflammatory, antioxidant, antibiotic, and lipid lowering agents. Future research efforts, and particularly randomised clinical trials, need to examine ways of modifying the links between inflammation, atherosclerosis, and thrombosis.

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