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The role of inflammation in atherothrombosis: implications for clinical practice

Alain Tedgui

Abstract: Inflammation plays a key role in atherothrombosis: in the development of plaques, plaque rupture and thrombus formation. Various biochemical substances have been shown to be involved in the inflammatory process, some with pro-inflammatory activity and others with anti-inflammatory activity. Increased expression of many inflammatory mediators (e.g. C-reactive protein, CD40 ligand, P-selectin and IL-6) has been shown to correlate with increased risk of atherothrombotic events. One possible strategy for primary and secondary prevention is likely to focus on minimizing the inflammatory response and tipping the balance in favour of anti-inflammatory mediators and, therefore, plaque stability.

Key words: cytokines; immunology; inflammation; molecular risk factors; plaque rupture; platelets

Introduction

Adverse vascular events such as myocardial infarction (MI), stroke and peripheral arterial disease (PAD) are the most common causes of mortality and morbidity in the Western world today. Epidemiological studies indicate that the prevalence of vascular disease is increasing on a global basis and is likely to reach epidemic proportions in the coming decades.^{1–3}

Atherothrombosis is the pathological process underlying adverse vascular events and involves the complex sequence of events leading to atherosclerotic plaque development, plaque rupture and thrombus formation.⁴ It is this final stage in the process that is responsible for clinically observable thrombotic events and adverse vascular outcomes in cardiovascular, cerebrovascular and peripheral vascular beds. Studies indicate that, in patients with atherothrombotic disease, plaque formation is likely to be widespread throughout the vasculature, often affecting more than one vascular bed.^{5,6}

Over the last few years, increasing evidence has emerged that inflammation plays a key role in the development of atherosclerotic plaques, in plaque rupture and thrombus formation.^{7,8} As yet, our understanding of these processes is not fully developed,

although recent discoveries in vascular biology continue to add to our knowledge base. Nevertheless, as we continue to learn more about the role played by inflammation in the atherothrombotic process, existing knowledge can be applied to the clinical management of cardiovascular disease.⁹ Future research is likely to increase our understanding of the biochemistry underlying atherothrombosis and will continue to have important implications for current and future therapeutic approaches.

This article seeks to explore recent developments in elucidating the role of inflammation in the development of atherothrombosis and to consider the implications for the clinical management of patients with atherothrombotic disease.

Role of inflammation in the pathogenesis of atherothrombosis

Plaque formation and rupture is a complex process involving many inflammatory cell types and numerous biochemical mediators. The process is a dynamic one with plaque structure and composition responding to the local environment.¹⁰ Plaques can exist as stable structures or may be unstable and prone to rupture. While stable plaques are relatively harmless, unstable plaques lead to atherothrombotic events and adverse vascular outcomes.¹¹

At the very early stages of plaque formation, inflammation of the endothelium occurs. Lipoprotein accumulation in the arterial wall is observed, probably as a consequence of high circulating levels of cholesterol and oxidation of low-density lipoprotein (LDL).

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Recruitment of monocytes and T-lymphocytes follows, mediated by specific leukocyte adhesion molecules expressed on the surface of the vascular cells. Monocytes and T-lymphocytes then penetrate into the arterial intima.¹² This early recruitment of inflammatory cells leads to a complex cascade sequence of expression of inflammatory mediators, which promote the inflammatory process.

The next stage in the process of plaque formation is the differentiation of monocytes into macrophages and the development of foam cells consequent to the ingestion of cholesterol by macrophages. This is followed by migration of smooth muscle cells into the developing plaque and the secretion of collagen, which aids in the formation of a fibrous cap.¹³

Atherosclerotic plaques can continue to grow and develop over significant periods of time. Providing the plaque structure remains stable, rupture is unlikely and therefore without significant clinical consequences.¹⁴ However, a shift in balance to increased inflammatory activity can lead to changes in plaque structure that confer instability.¹⁵ Development of plaque instability generally involves growth of the lipid core, renewed inflammatory response, macrophage activation, secretion of inflammatory mediators, secretion of matrix metalloproteinases (MMPs) that encourage the degradation of collagen and, finally, weakening of the fibrous cap.^{13,16}

Sudden plaque rupture occurs either as a consequence of rupture of the plaque's fibrous cap or superficial erosion of the intimal surface. Both surface erosion and fibrous cap rupture are generally followed by thrombus formation.¹⁷ Superficial plaque erosion with consequent exposure of the subendothelial collagen promotes platelet aggregation. Once a thrombus is formed, activated platelets promote monocyte adherence to the underlying endothelium and there is active synthesis by platelets of factors that potentiate inflammation. This process promotes further inflammatory activity contributing to further plaque destabilization.¹⁸

Inflammatory markers and mediators involved in plaque development and rupture

A large number of biochemical molecules of various kinds have been shown to be involved in the inflammatory process.¹⁹ These include cytokines, adhesion molecules, CD40/CD40 ligand, metalloproteinases, acute phase proteins, asymmetric dimethylarginine (ADMA), isoprostanes, tissue factor and plasminogen activator inhibitor-1 (PAI-1). Some studies have also demonstrated an association between coronary artery disease and increased plasma levels of oxidized LDL.²⁰ Furthermore, antibodies to oxidized LDL have been shown to predict MI and progression of carotid atherosclerosis in non-autoimmune subjects.²¹ Table 1 lists some of the main mediators that have been

identified as having an association with plaque development and rupture. For some of these substances the precise role played in the plaque development process has been established, while for others it remains obscure. Some mediators appear to have predominantly pro-inflammatory activity, while others are anti-inflammatory in nature. The nature and progress of an atherosclerotic plaque appears to depend upon the prevailing balance of pro- and anti-inflammatory activity.²²

Cytokines

The cytokine family is probably the most studied group of inflammatory mediating molecules and includes lymphokines (e.g. macrophage activating factor [MAF], macrophage migration inhibition factor [MIF]), interleukins (e.g. IL-1, IL-2, IL-6, IL-10, IL-12, IL-18), tumour necrosis factor (TNF- α), chemokines (e.g. IL-8, monocyte chemoattractant protein 1 [MCP-1]) and interferon (IFN- γ). Cytokines play a number of different roles in the development of inflammation, including controlling the duration and nature of the immune response and the remodelling that accompanies inflammation.²³ Cytokines exert their effects by facilitating inter-cell signalling. Some cytokines are chemoattractant (e.g. MCP-1) and facilitate the movement of inflammatory cells within the developing plaque;²⁴ others stimulate the production of acute phase proteins.²⁵ Animal studies have shown that cytokines such as IL-10²⁶ and transforming growth factor (TGF- β)²⁷ have anti-inflammatory properties and inhibit the production of pro-inflammatory cytokines. Anti-inflammatory cytokines have been shown to have anti-atherogenic properties.²²

TNF- α is a potent inflammatory cytokine. TNF receptor 1 and TNF receptor 2 are both soluble receptors shed by the many cell types on which they reside. TNF- α promotes the expression of adhesion molecules such as ICAM-1 on the endothelium, facilitating leukocyte adherence and migration from capillaries into the brain, microvessel occlusion, and subsequently a progressive reduction in blood flow.²⁸ Elevation of TNF- α and TNF receptor levels occurs in a variety of infectious, inflammatory, autoimmune, and neoplastic diseases. Elevated levels of TNF receptor may be a reflection of the inflammatory mechanisms operative in the atherosclerotic plaque.²⁹

IL-6 is a multifunctional proinflammatory and vasodepressor cytokine that mediates both immune and inflammatory responses. Produced in response to several factors, including IL-1, IFN- γ and TNFs, it is the primary determinant of C-reactive protein (CRP) release from the liver and the only substance known to induce synthesis of all acute phase proteins.³⁰ IL-6 gene transcripts are known to be expressed in human atheromatous lesions.³¹ Production of inflammatory markers is stimulated by circulating cytokines such as IL-6 and TNF- α , which in turn may also be generated

Table 1 Pro- and anti-inflammatory markers and mediators involved in plaque development and rupture.

Marker/mediator	Localization/expression	Physiological function/role
Cytokines: e.g. IL-1, IL-6, IL-8, IL-12, IL-18, TNF- α , IFN- γ (pro-inflammatory)	Macrophages, Th1 lymphocytes	Control duration and nature of immune response and remodelling that accompanies inflammation by inter-immune cell signalling; effects may be pro- or anti-inflammatory
IL-10, TGF- β (anti-inflammatory)	Macrophages, Th2/Th3 lymphocytes	
Adhesion molecules: e.g. ICAM-1, VCAM-1, P-selectin, E-selectin	ICAM-1, VCAM-1 and E-selectin are expressed by endothelial cells; P-selectin is expressed by both endothelial cells and by platelets	Mediate contact of inflammatory cells with other cells (e.g. endothelial cells)
CD40/CD40 ligand	T-lymphocytes and platelets	Multifunctional signalling molecule
Metalloproteinases	Macrophages, smooth muscle cells	Tissue remodelling
Acute phase proteins: e.g. CRP, fibrinogen, serum amyloid A	Synthesized in the liver; some evidence for local vascular synthesis of CRP	Multiple effects (e.g. induction of adhesion molecule expression)
ADMA	Inhibitor of nitric oxide synthase	Increased levels associated with endothelial dysfunction
Isoprostanes	Synthesized by free radical-induced peroxidation of arachidonic acid	Markers of lipid peroxidation
Tissue factor	Present in atherosclerotic plaques	Involved in plaque thrombogenicity
PAI-1	Serine protease inhibitor	Impairs fibrinolysis

IL, interleukin; TNF- α , tumour necrosis factor alpha; IFN- γ , interferon gamma; TGF- β , transforming growth factor beta; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule. CRP, C-reactive protein; ADMA, asymmetric dimethylarginine; PAI-1, plasminogen activator inhibitor-1.

from a variety of systemic sources, including adipose tissue, which is a potent source of cytokines, and inflammatory cells either in the atherosclerotic lesion in the arterial wall or elsewhere.

Adhesion molecules

Adhesion molecules shed from a variety of cells give rise to soluble forms measured in the plasma. Adhesion molecules such as ICAM-1 vascular cell adhesion molecule (VCAM-1) and E-selectin are expressed by endothelial cells and play a role in facilitating the contact between inflammatory cells and endothelial cells.³² Soluble VCAM-1 (sVCAM-1) is another well-defined soluble adhesion molecule which participates actively in the inflammatory-endothelial adhesive interaction process in atherothrombosis.³³ Both sVCAM-1 and sICAM-1 have been used as noninvasive markers for the evaluation of endothelial dysfunction.

Available evidence suggests a role for the selectins in the early stages of atherogenesis. E-selectin is synthesized *de novo* by endothelial cells when activated by IL-1 or TNF- α .³⁴ P-selectin, an adhesion molecule expressed by platelets, facilitates the anchoring of inflammatory cells to the platelet and to endothelial cells.³⁵ E-selectin and P-selectin are preferentially

expressed in the endothelium overlying atherosclerotic plaques.³⁶

CD40/CD40 ligand

CD40 ligand (CD40L), a key signalling molecule that has many functions in the immune system, is a cell surface molecule and is expressed by activated T-lymphocytes and platelets. CD40 ligand is released by activated platelets during thrombus formation.³⁷ CD40L also exists in a soluble form (sCD40L), which is shed from lymphocytes and platelets within minutes to hours after cell activation.³⁸ Studies have shown that CD40L can interact with endothelial cells, smooth muscle cells and macrophages to promote plaque instability.³⁹ The precise way in which released CD40L affects plaque composition is unclear; however, studies have demonstrated that antibody blockade of CD40L in apoE $^{-/-}$ mice leads to an increase in plaque stability.⁴⁰

Metalloproteinases

The MMP family of enzymes plays a key role in tissue remodelling.⁴¹ They can also act as regulatory molecules by processing matrix proteins, cytokines, growth factors and adhesion molecules to generate

fragments with enhanced or reduced biological effects. Leukocytes, particularly macrophages, are a major source of MMP production, and MMP release by leukocytes plays a vital role in allowing leukocytes to extravasate and penetrate tissues.

It is proposed that infiltrating inflammatory cells interact with the extracellular matrix and oxidized lipoproteins, contributing to increased production of MMP-9 by macrophages.⁴² The presence of increased macrophages provides stimulus for synthesis or activation of MMPs by neighbouring cells facilitating further structural changes and advancement of atherosclerotic lesions.⁴³ The MMPs contribute to the development of de novo atherosclerotic plaque as well as to the rupture of these plaques by degrading the associated extracellular matrix.

Acute phase proteins

Acute phase proteins (e.g. CRP, fibrinogen and serum amyloid A) are primarily produced in the liver and are indicative of inflammation at sites distant from the liver. However, recent evidence suggests that CRP may also have a direct role in the pathogenesis of atherosclerotic lesion formation.^{44,45} CRP appears to affect a number of interrelated pathways in the vascular endothelium, including the induction of expression of adhesion molecules, foam cell formation and monocyte recruitment in the arterial wall.⁴⁴

Blood viscosity depends mainly on the amount of blood cell content and plasma proteins, mainly fibrinogen. Thrombin converts fibrinogen into fibrin, which, after polymerization, converts into cross-linking fibrin. The presence of fibrin monomer is considered to indicate a prothrombotic state.⁴⁶

ADMA

ADMA is an endogenous inhibitor of nitric oxide (NO) synthase, a crucial enzyme in the maintenance of vascular tone and structure. Ito et al.⁴⁷ showed that incubation of cultured human endothelial cells with oxidized LDL or TNF- α increased the level of ADMA in the conditioned medium. ADMA is metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), and inhibition of DDAH activity has been shown to lead to increased ADMA levels and endothelial dysfunction.

Isoprostanes

Isoprostanes are a complex family of compounds produced from arachidonic acid via a free-radical-catalysed mechanism. F₂-isoprostanes are prostaglandin-F₂-like compounds generated by free radical-induced peroxidation of arachidonic acid⁴⁸ and are used extensively as clinical markers of lipid peroxidation in cardiovascular disorders. Lipid oxidation has been shown to be a prominent feature of atherosclerosis. The presence of isoprostanes and other lipid oxidation products has been

clearly demonstrated in lesions.⁴⁹ Quantification of isoprostanes provides a non-invasive approach for studying lipid peroxidation in vivo.

Tissue factor

Tissue factor is released in the bloodstream once an atherothrombotic plaque is disrupted, triggering the formation of a superimposed thrombus that leads to vessel occlusion and subsequent ischemic symptoms distal to it.⁵⁰ Specific inhibition of the tissue factor pathway by its physiologic inhibitor, tissue factor pathway inhibitor (TFPI), significantly reduces plaque thrombogenicity.⁵¹

PAI-1

PAI-1 is a serine protease inhibitor whose major function is to negate plasminogen activation and impair fibrinolysis.⁵² It is present in plasma and tissues. Circulating PAI-1 levels are thought to play an important role in the development of atherothrombosis by decreasing fibrin degradation. It may be that increased PAI-1 in blood might facilitate persistence of microthrombi and a prothrombotic state, resulting in attenuation of thickness of elaborated fibrous caps implicated in the vulnerability of atheroma and rupture.

Mediators and markers as predictors of cardiovascular risk

Increased expression of many of these inflammatory substances has been shown to be correlated with an increased risk of atherothrombotic events in a number of studies. Increased expression can be identified as either an elevated local concentration of a substance or raised systemic levels. Substances that have a clear role in the inflammatory response can be regarded both as mediators and markers of inflammation. Substances with an established relationship between increased expression and increased cardiovascular risk but an unclear basis of correlation should be regarded only as markers for inflammation.

Correlation between systemic levels of a substance and the development of vascular disease has important clinical implications as measurement of these substances may be a useful means of predicting cardiovascular risk. Accurate prediction of cardiovascular risk has the dual benefit of providing the physician with a means of assessing a patient's pharmacological requirements and assessing the response to therapy.

A clear correlation between elevated plasma levels and increased risk of vascular disease has been demonstrated for CRP in males,⁵³ CD40 ligand,⁵⁴ E-selectin,⁵⁵ IL-6,⁵⁶ TNF- α ⁵⁷ and ICAM-1.⁵⁸ Of the substances shown to correlate with vascular disease risk, CRP has been the most widely studied, despite

the fact that the relationship between the elevation of circulating levels of CRP and increased risk remains poorly understood.

The fact that plasma concentration of CRP predicts the risk of future MI and stroke was identified in a study in men as long ago as 1997⁴⁴ and subsequent studies in men and women have confirmed the correlation between cardiovascular risk and CRP levels.^{59,60} Elevated baseline CRP levels have also been shown to predict a heightened risk of 30-day death or MI after coronary intervention.⁶¹

Recently, the relationship between CRP and the Framingham Coronary Heart Disease Risk Score (FCRS) has been studied in a cross-sectional survey of 1666 individuals free of cardiovascular disease.⁶² Among men and women not using hormone replacement therapy (HRT), CRP levels were significantly related to 10-year FCRS categories although correlated only minimally with individual components of the FCRS. Surprisingly, for women taking HRT, no significant relationship was observed between CRP and the FCRS, although the power to detect effects in this subgroup was considered by the investigators to be limited.

Circulating CRP levels are affected by a number of factors associated with cardiovascular disease, including demographic parameters, lifestyle and pharmacological therapy. CRP levels are decreased by weight loss, exercise and therapies such as lipid-lowering drugs and antiplatelet agents, while CRP levels are increased by smoking, diabetes, elevated triglyceride levels and high blood pressure.⁶³ It remains unclear, however, whether the relationship between increased circulating CRP levels and increased cardiovascular risk is a causal or casual relationship. Furthermore, CRP levels are also influenced by chronic fatigue, coffee consumption, sleep disturbances and depression, which are not traditionally considered to have an influence on cardiovascular risk. Therefore, while it is comparatively simple to make risk predictions based on CRP levels on a population basis, it may be more difficult to interpret CRP levels in the individual patient.

The American Heart Association/Centers for Disease Control (AHA/CDC) workshop assessed the value of various markers for routine risk assessment.⁶³ A recent clinical study, published after the workshop, reported that the CRP concentration is a relatively moderate predictor of the risk of coronary heart disease and adds only marginally to the predictive value of established risk factors for coronary heart disease⁶⁴ and similar overall findings were observed in an updated meta-analysis involving a total of 7068 patients with coronary heart disease,⁶⁴ suggesting that CRP may be a relatively moderate predictor of coronary heart disease. It must be noted though that the clinical study mentioned above involved patients from The Reykjavik Study, which reflects an Icelandic population. Therefore, although the relative homogeneity

of the Reykjavik population should have minimized certain residual biases, further clarification of the predictive value of CRP in coronary heart disease in the general population will require pooling studies on the basis of data for individual participants from each of the available prospective studies.

The participants of the AHA/CDC workshop concluded that to be useful in regular clinical practice, a marker would have to be independent from established risk factors, be clearly correlated with clinical endpoints with data available on normal population values, improve the overall risk prediction beyond traditional factors, be a generalization across various populations and have an assay that could be standardized with an acceptable cost. Of the currently available assays for inflammatory markers, only the high sensitivity assay for CRP (hsCRP) was considered to meet these criteria. The participants recommended that relative risk be categorized as low, average or high corresponding to approximate tertiles of values: <1.0, 1.0–3.0 and >3.0 mg/l, respectively. However, the workshop also asserted that, for primary prevention of coronary disease, CRP should only be used as an adjunct to the major risk factors to further assess absolute risk, while, for secondary prevention, intensive treatment was required regardless of CRP levels.

TNF- α has also been associated with an elevated risk of recurrent MI and cardiovascular death after a first MI.⁵⁷ TNF- α levels were correlated with the ankle-brachial index.⁶⁵ Other investigators have suggested that soluble TNF receptor levels may be a better marker of atherosclerotic burden than TNF- α itself. A study that sought to determine whether TNF- α and TNF receptor levels were associated with carotid plaque thickness concluded that relative elevation in TNF receptor levels, but not TNF- α , was associated with carotid atherosclerosis among individuals aged <70 years, suggesting that TNF receptors may be a more stable marker of inflammatory burden than TNF- α .²⁹

Other inflammatory markers have also been related to cardiovascular risk. Among apparently healthy men in the Physicians' Health study, baseline levels of IL-6 were independently predictive of risk of future MI.⁵⁶ Soluble ICAM-1, known to mediate leukocyte adhesion and transmigration across the vascular wall, has been found to be higher in patients with stable angina, unstable angina, and acute MI compared with control patients.⁶⁶ Higher levels of E-selectin have been found with elevations of ICAM-1 in patients with either coronary heart disease or carotid artery atherosclerosis compared with control patients.⁵⁵ In a small study, soluble fibrin monomer complex was related to the development of MI in young patients.⁴⁶ Therefore, fibrin may be a candidate for an early marker for thrombosis, but further studies are needed to evaluate the contribution of this factor to cardiovascular disease.

Elevated blood levels of sCD40L appear to be associated with the risk of future cardiovascular events in otherwise healthy women and predict patients with high-risk atherosclerotic lesions.^{67,68} High plasma levels of fibrinogen are also associated with increased risk of cardiovascular disease.⁶⁹ In the Caerphilly and Speedwell Collaborative Heart Disease Studies, fibrinogen, plasma viscosity and white blood cell count were independent risk factors for ischemic heart disease.⁷⁰

ADMA has also been shown to be increased in the plasma of humans with hypercholesterolemia or atherosclerosis. A study assessing the progression of the intima-media thickness during a period of 1 year found that ADMA and CRP levels emerged as the sole independent predictors of the progression of intimal lesions in patients with initially normal intima-media thickness.⁷¹ A prospective, nested, case-control study has demonstrated that middle-aged men who did not smoke, but who had ADMA plasma levels in the highest quartile, had a 3.9-fold increased risk for acute coronary events compared with the other quartiles.⁷² Further trials are warranted to prove the hypothesis that ADMA is a predictor of cardiovascular events and/or mortality, and thus to assess its suitability as a diagnostic marker of ischemic vascular disease.

Isoprostanes have been detected in human atherosclerotic plaque⁷³ and levels of isoicosanoids, free radical-catalysed isomers of the traditional enzymatic products of arachidonic acid, are higher in patients with symptomatic plaques than in their asymptomatic peers,⁷⁴ suggesting an association with plaque instability.

Circulating tissue factor has been associated with increased blood thrombogenicity in patients with unstable angina and chronic coronary artery disease,⁷⁵ and there is evidence that acute thrombosis may be initiated by membrane-bound circulating tissue factor originating from activated or injured cells.⁷⁶ Elevated levels of PAI-1 have been linked to increased cardiovascular risk in middle-aged men⁶⁷ and circulating PAI-1 concentrations are elevated in young men at increased risk for recurrent infarction.⁵² In the Physicians' Health Study,⁷⁷ it appeared that increased EPA activity was associated with increased coronary events.

Implications for therapy

The recent upsurge in our understanding of the role played by inflammation in the atherothrombotic process has significant implications for current and future therapeutic approaches to the primary and secondary prevention of atherothrombotic events.

There is evidence that drugs currently employed for primary and secondary prevention of cardiovascular disease may have anti-inflammatory effects over and

above their primary pharmacological effects. Drugs such as ACE inhibitors⁷⁸ and statins⁷⁹ appear to exert beneficial effects even in patients with normal blood pressure or lipid levels. These observations have led to the suggestion that these drug classes possess therapeutic benefits beyond their antihypertensive or lipid-lowering effects.

Evidence that statins may modulate inflammatory processes has been derived from studies that have shown that statins can decrease expression of IL-6, IL-8, MCP-1, CRP, TNF- α and MMP.^{80,81} Statins have also been shown to prevent the activation of monocytes into macrophages and inhibit the production of cellular adhesion molecules.⁸² Similarly, the antidiabetic agent rosiglitazone has been shown to reduce circulating levels of inflammatory markers, suggesting an anti-inflammatory effect of the drug over and above its key pharmacological effect.⁸³

Antiplatelet agents have also been shown to have the potential to modify inflammatory processes. *Ex vivo* studies have demonstrated that clopidogrel treatment, with or without acetylsalicylic acid (ASA), abolishes the ADP-induced expression of CD40L in human platelets.⁸⁴ Clopidogrel (but not ASA) has also been shown to reduce P-selectin expression and formation of platelet-leukocyte aggregates in patients with atherothrombotic disease⁸⁵ and in patients with an acute coronary syndrome.⁸⁶ Furthermore, CRP levels have been shown to be significantly lower 7 days after the event in acute ischaemic stroke patients treated with clopidogrel compared with those receiving heparin treatment,⁸⁷ while clopidogrel pretreatment prior to percutaneous coronary intervention (PCI) has also been shown to attenuate the periprocedural rise in CRP.⁸⁸ Other anti-inflammatory activities associated with clopidogrel treatment include decreased serum levels of soluble ICAM-1 seen after treatment of healthy volunteers with the drug⁸⁹ and the reduced periprocedural expression of P-selectin in PCI patients pretreated with clopidogrel.⁹⁰

However, it is unclear whether these anti-inflammatory actions play a significant role in the reduction of cardiovascular risk that these therapies afford and further studies are required to explore these interesting observations. No currently available therapies have convincingly demonstrated a cardiovascular protective effect on the basis of their anti-inflammatory activity.

In the future, the development of new therapies designed for the primary and secondary prevention of atherothrombotic events is likely to focus on strategies that decrease the inflammatory response and tip the balance in favour of anti-inflammatory mediators and, therefore, plaque stability. Approaches may include the development of agents that block pro-inflammatory cytokine signalling, agents that augment the anti-inflammatory activity of other cytokines and agents that either block the transcription of

inflammatory mediating molecules or upregulate anti-inflammatory molecules.

Conclusion

As more is discovered about the complex sequence of inflammatory events involved in plaque formation and rupture, vascular medicine will reap the benefits. Future research should enable improved risk assessment and the development of new therapeutic strategies that target the biochemical perturbations that underlie atherothrombosis.

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Note in proof

After this manuscript was accepted for publication, two articles published in the January 6, 2005 issue of the *New England Journal of Medicine*, one by Nissen et al. and one by Ridker et al., confirmed that reducing CRP levels through the use of statin therapy improves the clinical outcome independently of the reduction in serum LDL cholesterol levels.^{91,92}

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