Inflammatory mechanisms in atherosclerosis: from laboratory evidence to clinical application

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From the initial stages of leukocyte recruitment to diseased endothelium, to eventual rupture of unstable atheromatous plaque, pro-inflammatory mechanisms mediate key steps in atherogenesis and its complications. Lipid lowering, both with diet and statin therapy, has been shown to have favorable effects on inflammatory processes in atheromatous plaque. Several plasma markers of inflammation have been found to predict future cardiovascular risk, both among patients with acute coronary syndromes and myocardial infarction, and among healthy men and women. C-reactive protein (CRP), a pattern recognition molecule linked to the innate immune system, is a sensitive marker of low-grade vascular inflammation, which may also have direct pro-inflammatory actions. Recent studies have shown that statin therapy may lower CRP levels independent of lipid-lowering effects. Statin therapy may also be highly effective for the prevention of cardiovascular events among individuals with elevated CRP levels. The role of statin therapy for plaque stabilization in acute coronary syndromes, and for prevention of future plaque rupture among healthy individuals with evidence of vascular inflammation, is an area of active research.

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Introduction

Accumulating evidence suggests that inflammatory processes play a key role in the pathogenesis of atherosclerosis[1]. Atheromatous plaque is covered by a fibrous cap, which separates the pro-thrombotic *milieu* of the lipid pool from the luminal blood flow. Unstable plaques are characterized by excessive inflammation which overwhelms the plaque's ability to repair. Rupture of the fibrous cap with the resultant release of pro-thrombotic tissue factor may herald the onset of an acute ischemic event. Several plasma markers of inflammation have been found to predict subsequent cardiovascular risk, both among patients with acute coronary syndromes[2-6] and myocardial infarction[7], and also among healthy men and women[8-18]. Emerging evidence suggests that statin therapy may have potent anti-inflammatory effects[19], and that plaque stabilization with statin therapy may prove to be an important intervention for patients with unstable coronary syndromes, and those at risk for cardiovascular events.

Inflammation in atherosclerosis: laboratory insights

The initial phases of atheroma development are marked by the adherence and subsequent transmigration of leukocytes into the subintima. This process is facilitated by molecules such as intercellular adhesion molecule-1 (ICAM-1) and E-selectin, which are produced by endothelial cells in response to stimulation by the pro-inflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF)[20]. Cytokine-stimulated endothelial cells also produce monocyte chemotactic protein-1, monocyte colony stimulating factor, and IL-6 which further amplifies the inflammatory cascade[21]. The cytokine-induced endothelial synthesis of these pro-inflammatory agents is mediated by the transcription factor NF-κB[22].

Vulnerable plaque is characterized by a thin fibrous cap and a large lipid core[23]. The key component of the fibrous cap which confers stability and tensile strength is interstitial collagen. Macrophages, in response to stimulation by T cells, produce matrix metalloproteinases (MMP) such as MMP-1 and MMP-13, which actively break down collagen. T cells may also signal to smooth muscle cells to decrease synthesis of new collagen by the production of cytokines such as interferon-γ[24]. Conversely platelet-derived growth factor, released by platelets during thrombosis, and transforming growth factor-β increase the rate of collagen synthesis. Thus, a dynamic

equilibrium exists between collagen synthesis by smooth muscle cells and collagen breakdown by degrading enzymes. If the balance is tipped in favor of a pro-inflammatory state, fibrous cap thinning and eventual rupture may ensue.

The adverse consequences of plaque rupture are mediated by thrombus formation. Tissue factor, over-expressed by endothelial cells and macrophage foam cells, is a crucial initiator of thrombosis. Endothelial cells produce tissue factor in response to stimulation by IL-1 and TNF, but the stimulus for macrophage production has been unclear. Recent work has shown that a CD40 ligand (CD154) can stimulate tissue factor expression by binding to its receptor CD40 on leukocytes[25]. Platelets express CD154[26], which illustrates the potentially important interaction between pro-thrombotic and pro-inflammatory mechanisms[27]. Moreover, it has recently been shown that leukocyte binding and migration across a carpet of platelets adherent to diseased or injured intima, is dependent on the leukocyte integrin Mac-1 and platelet glycoprotein 1bα[28].

Lipid lowering has been shown to favorably influence atheromatous plaque composition. Rabbits fed with a high cholesterol diet develop atheromas with a high concentration of macrophages in the lipid core. These macrophages overexpress MMP-1, the critical rate-limiting enzyme in collagen breakdown. In rabbits switched to a low-cholesterol diet, inflammatory cells and levels of MMP-1 are markedly reduced[29]. Statin therapy has also been shown to have beneficial effects on plaque composition. In the rabbit model, statins have also been shown to diminish macrophage accumulation, MMP-1 expression, and increase type 1 procollagen synthesis by smooth muscle cells[30]. Recently, similar changes have been observed in human carotid plaque following pravastatin therapy[31].

A potential role for the innate immune system?

C-reactive protein (CRP) is thought to play a role in the innate immune system, where it acts as a pattern recognition molecule which rapidly activates the immune response[32]. Similar to immunoglobulin G, CRP activates complement[33], binds to Fc receptors and acts as an opsonin for various pathogens. Produced mainly in the liver in response to IL-6, CRP also stimulates monocyte release of inflammatory cytokines such as IL-1β, IL-6 and TNF-α[34]. CRP may directly act as a pro-inflammatory stimulus to phagocytic cells by binding to the FcγRII receptor[35], and CRP has recently been localized directly within atheromatous plaques where it precedes and mediates monocyte recruitment[36]. CRP also causes expression of ICAM-1 and vascular cell adhesion molecule-1 by endothelial cells[37]. Furthermore, it has recently been demonstrated that CRP opsonization of low-density lipoprotein (LDL) mediates LDL uptake by macrophages[38].

Accumulating evidence suggests that plasma levels of CRP are a sensitive marker of chronic low-grade vascular inflammation and may help predict which individuals are at increased risk for future ischemic events. Interestingly, given its pivotal role in the pro-inflammatory atherogenic pathway, NF-κB also plays an important role in the innate immune response, regulating the transcriptional activation of cytokines, including TNF-α and IL-1[39].

Clinical evidence for inflammatory mechanisms in atherosclerosis

Given the accumulating data that collagenolytic enzymes and cytokines secreted by pro-inflammatory cells can degrade the fibrous cap and transform the endothelium into a pro-adhesive and pro-coagulant agent, attention in clinical studies of acute coronary syndromes has focused on the role of inflammation. Pioneering work from investigators in Rome has shown that patients with unstable angina who have elevated blood levels of CRP (> 3 mg/l) have a higher rate of death, acute myocardial infarction, and need for revascularization procedures compared to patients with CRP levels < 3 mg/l[2]. Moreover, patients with acute myocardial infarction show a rise in CRP levels within 6 hours of symptom onset, suggesting that the rise in CRP levels may not be due to myocardial necrosis, but rather may be secondary to an underlying pro-inflammatory state. This concept is supported by the observation that patients with vasospastic angina have persistently normal CRP levels, despite frequent episodes of ST segment elevation[40]. Further evidence in support of a role for inflammatory processes in unstable coronary syndromes comes from data showing that increased levels of IL-1 receptor antagonist and IL-6 at 48 hours after admission are associated with a complicated hospital course[41].

These workers have also recently reported that at discharge elevated levels of CRP in patients with unstable angina predict an increased risk of cardiovascular events over the following year[3]. Furthermore, CRP has been shown to predict the recurrence of ischemic events in patients undergoing coronary artery bypass grafting[42] and also early adverse events and late restenosis in patients undergoing percutaneous transluminal coronary angioplasty[43].

These findings, allied to the salutary effects that statins have been shown to have on plaque inflammation, have supported clinical trials of early statin therapy among patients with acute coronary syndromes. The results of the recent Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) study suggest that early therapy with atorvastatin may indeed reduce recurrent ischemic events within 16 weeks in patients with acute coronary syndromes[44]. The potential role of statin therapy for plaque stabiliza-

tion in patients with acute coronary syndromes is an area of ongoing research.

Given that the development of atherosclerosis is a likely chronic low-grade inflammatory condition, attention has also focused on whether plasma markers of inflammation can predict future cardiovascular events among healthy individuals. Plasma levels of ICAM-1[16], P-selectin[15], IL-6[13,14] and CRP[8-10,12,15,17,18] have each been shown to predict cardiovascular risk in healthy populations. Of these, CRP has been the most extensively studied with consistent evidence from US and European studies suggesting that baseline levels of CRP are a strong independent predictor of future cardiovascular risk. Indeed in a large cohort of healthy US women, of a wide variety of lipid and inflammatory risk factors studied, CRP was the strongest predictor of future cardiovascular risk[17]. Moreover the addition of CRP evaluation to standard lipid testing significantly improved risk prediction at all lipid levels, and even among women with low LDL levels (< 130 mg/dl), elevated CRP levels identified those at high risk for future cardiovascular events (Fig. 1).

Data from the Cholesterol and Recurrent Events (CARE) trial first suggested that statin therapy may lower CRP levels and that this effect is independent of lipid lowering[45]. This finding has been substantiated in more recent studies[46-48]. Furthermore, intriguing data from the CARE trial suggested that the benefit of pravastatin therapy in the prevention of future cardiovascular events is greatest among those with persistent inflammation, as evidenced by elevated CRP and serum amyloid A levels[7].

This concept has recently been tested in an analysis of the Airforce/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) population[48], a large primary prevention cohort randomized to lovastatin or placebo[49]. The study population was divided into four groups according to the median LDL (149

Figure 1. *Predictive value of inflammatory markers among women with low-density lipoprotein levels < 130 mg/dl. CRP = C-reactive protein; IL-6 = interleukin-6; SAA = serum amyloid A; sICAM-1 = soluble intercellular adhesion molecule-1. From Ridker et al.[17], modified.*

mg/dl) and CRP (0.16 mg/dl) levels[48]. Individuals with low LDL and low CRP levels were at low risk of future cardiovascular events, and showed no benefit from statin therapy. Those with high LDL levels (> 149 mg/dl) were at more than 2-fold increased risk irrespective of CRP levels, and benefited substantially from statin therapy. The most interesting group, however, was that which consisted of those individuals with LDL levels < 149 mg/dl but with elevated CRP levels. These individuals were at more than 2-fold increased risk of future cardiovascular events, and derived a large benefit from statin therapy, similar to those with high LDL levels.

These results suggest that testing for CRP levels in primary prevention may identify individuals without overt hyperlipidemia who are at high risk of future cardiovascular events. Furthermore, CRP testing may guide the targeted use of statins among patients with lipid levels below current treatment guidelines, who may benefit substantially from statin therapy. These findings require confirmation in future randomized clinical trials designed to directly address this hypothesis. Ongoing studies from our group suggest that the projected life expectancy benefits with a strategy of CRP testing to target statin therapy for primary prevention among individuals without overt hyperlipidemia may be substantial. Data regarding the cost-effectiveness of such a strategy are clearly required, and such analyses are ongoing.

Conclusion

Accumulating evidence confirms a pivotal role for inflammatory processes in the pathogenesis of atherosclerosis and its complications. CRP, a pattern recognition molecule linked to the innate immune system, is a sensitive marker of vascular inflammation and may directly contribute to the inflammatory process by complement activation and by stimulating the release of pro-inflammatory cytokines. Recent work has confirmed that CRP is a strong predictor of future cardiovascular risk among healthy populations and among patients with unstable angina and acute myocardial infarction. Ongoing studies suggest that statin therapy may have potent anti-inflammatory effects, and may lower CRP levels. The role of statin therapy for plaque stabilization in acute coronary syndromes, and for the prevention of future plaque rupture among healthy individuals with evidence of vascular inflammation, is an area of active research.

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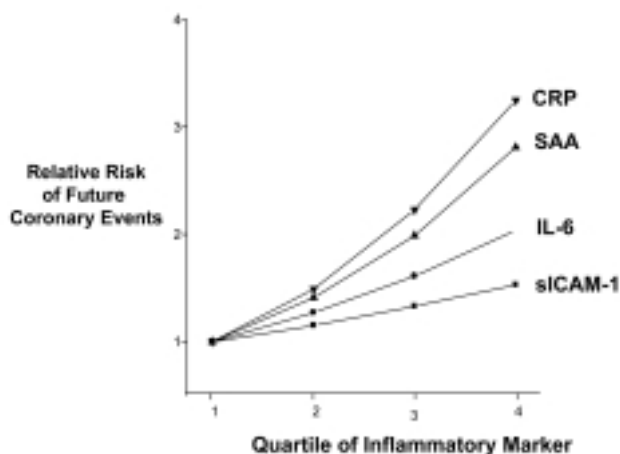2. Liuzzo G, Biasucci LM, Gallimore JR, et al. The prognos-

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