Inflammation in acute coronary syndromes

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The late Russell Ross's assertion that atherosclerosis is an inflammatory disease is now strongly supported by clinical, basic, and pathological research calling for an evolution in thought concerning the evaluation and treatment of acute coronary syndromes (ACS).[1-5] The initial insult is endothelial injury and subsequent dysfunction via the deleterious effects of the known cardiac risk factors such as oxidized LDL, infection, hyperglycemia, hypertension, hyperhomocysteinemia, or smoking. Irrespective of the cause of endothelial damage, the resultant activation and proliferation of inflammatory cells, smooth muscle cells, generation of cytokines, growth factors, and many other substances lead to the progression of atherosclerosis. The presence and degree of inflammation and procoagulant state, defined by elevated CRP, fibrinogen, interleukin (IL)-1, IL-6, TNF-α, adhesion molecules, plasminogen activator inhibitor (PAI-1), tissue factor, and composition of the atherosclerotic plaque have been strongly associated with an increased risk of future cardiac events.[6-9] Thus, the perpetuation of the inflammatory response likely plays a pivotal role in the pathobiology and vulnerability of the atherosclerotic plaque.

PATHOBIOLOGY OF INFLAMMATION, ATHEROSCLEROSIS, AND ACS

Endothelial function

The endothelium lies in a critical location between the remaining vascular wall and the circulating blood thereby functioning as the pivotal barrier that protects the arterial wall from injury. This critical monolayer of cells is pluripotential, carrying out the following functions: 1) provision of a nonthrombotic surface; 2) maintenance of vascular tone through the production and release of nitric oxide (NO), prostacyclin, and endothelin; 3) regulation of growth factors and cytokines; 4) provision of a nonadherent surface for leukocytes and platelets; and 5) the modification of lipoproteins as they transverse its permeable barrier.[5] Injury to this monolayer plays a key role in the initiation and progression of the atherosclerotic lesion by increasing adhesive cell surface glycoproteins such as vascular cell adhesion molecule-l (VCAM-1) and intracellular adhesion molecule (ICAM), adherence, migration, and activation of leukocytes, and smooth muscle cells, production of cytokines, chemokines, and growth factors, as well as the reversal from an antithrombotic to a prothrombotic state.[4,10-12]

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Adhesion molecules

Cell-cell interactions are a vital component in the pathogenesis of inflammation. Collectively known as cell adhesion molecules, three distinct families exist—the selectins, the integrins, and the immunoglobulin superfamily each with its own specific role in the inflammatory process. The process entails tethering and rolling of leukocytes on the activated endothelium, leukocyte activation, and ultimately firm adhesion and transendothelial migration along a chemotactic gradient generated by mediators of inflammation.[13,14]

Selectins are expressed on the cell surface of leukocytes (L-selectin), platelets (P-selectin), and endothelial cells (E-selectin). Upon activation from inflammatory cytokines, mainly TNF-α and IL-1, cell surface expression of each selectin is enhanced.[15-17] This process is vital in the early phase of inflammation mediating leukocyte recruitment and transient endothelial cell to leukocyte interactions (tethering and rolling phase). The subsequent steps of firm adhesion and migration of leukocytes is predominantly mediated through the interaction of integrins [leukocyte function associated antigen-1 (LAF-1), macrophage antigen-1 (MAC-1), very late activation antigen-4 (VLA-4) and GPIIb/IIIa receptor], the immunoglobulin superfamily [vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and intercellular adhesion molecule-2 (ICAM-2)] and potent stimulation by inflammatory cytokines including IL-1, IL-4, IL-8, TNF-α, INF-γ, and chemokines such as chemotactic protein-1.[13,14] In addition to the cell adhesion molecules on endothelial cells, leukocytes, and platelets, ICAM-1 and VCAM-1 are expressed on smooth muscle cells.[18] The interaction between leukocytes and smooth muscle cells contributes to smooth muscle cell migration and proliferation, cellular composition of the atherosclerotic plaque, and an increased expression of monocyte tissue factor mRNA, all of which are likely to be vital in influencing plaque stability.[18,19] An additional component that ties inflammation and the prothrombotic state involves the adhesion of activated platelets to the endothelium through the P-selectin-GPIIb/IIIa receptor interactions with subsequent platelet aggregation and thrombus formation.[20]

Growing evidence supports that the presence of increased cell adhesion molecules in serum or vascular tissue may reflect ongoing active vascular remodeling due to persistent inflammation. Elevated serum levels of the soluble form of the VCAM-1 receptor (sVCAM-1) has been associated with the extent of atherosclerosis in patients with peripheral vascular disease.[21] In patients with coronary artery disease, elevated levels of the soluble ICAM-1 (sICAM-1) has been found to be inversely proportional to HDL levels and associated with the presence of other coronary risk factors, unstable angina, myocardial infarction, and importantly to increased risk of future myocardial infarction in apparently healthy men.[22,23] Interestingly, immunohistochemical evaluation of coronary athrectomy tissue has shown P-selectin but not E-selectin, or ICAM-1 was expressed significantly greater in the setting of unstable angina versus stable angina.[24] This reflects an augmented response between an endothelial cell adhesion molecule and the activated platelet linking thrombus formation and unstable coronary syndromes.

Treatment strategies available based on the inhibition of cell to cell interactions have shown promise in the treatment of chronic inflammatory diseases, and recently coronary artery disease.[13,25] This should not be surprising given the marked similarities that exist between the pathophysiology of inflammatory diseases, such as rheumatoid arthritis, and atherosclerosis **(Table 1)**. ASA and other NSAIDS affect the expression and function of cell adhesion molecules, and have been shown to inhibit many phases of the adhesion cascade.[18] Direct antagonism via monoclonal antibodies and selectin-blocking agents against ICAM-1 and L-selectin has been shown to reduce neutrophil accumulation and myocardial injury in experimental animal studies.[26,27] New approaches using antisense oligonucleotides to inhibit mRNA translation for cell adhesion molecule expression, and inhibition of gene expression by synthetic DNA molecules and triplex-forming oligonucleotides have shown conceptual promise in animal studies.[13]

TABLE 1
SIMILARITIES BETWEEN ATHEROSCLEROSIS AND RHEUMATOID ARTHRITIS

	Atherosclerosis	Rheumatoid arthritis
Macrophage activation		
TNF-α	↑	↑
Metalloproteinases	↑	↑
Interleukin-6	UA ↑	↑
Mast cell activation	↑	↑
T-cell activation		
CD+DR+	UA ↑	↑
CD4+CD28–/INF+	UA ↑	↑
TH1/TH2 balance	TH1 ↑	TH1↑
B-cell activation	0 or ↑	0 or ↑
CRP	↑	↑↑
Adhesion molecules	↑	↑
Endothelin	↑	↑
Neoangiogenesis	↑	↑

Source: Modified, with permission, from Pasceri and Yeh, "A tale of two diseases: atherosclerosis and rheumatoid arthritis," Circulation 1999; 100(21):2124-2126.

CELLULAR AND HUMORAL MEDIATED RESPONSE

Monocytes and macrophages

Monocytes, the circulating precursors of tissue macrophages, are essential in the progression of atherosclerosis and are found in all stages of atherosclerotic lesions.[4,28] Their recruitment and infiltration through the endothelium into the intima are tightly coupled to the humoral activity of the T-lymphocyte. The colocalization of CD4+ T-cells and macrophages and the abundant expression of HLA II molecules in atherosclerotic lesions is strong evidence for the role of cell-mediated immunity in the development and progression of atherosclerosis. Population size of CD14dimCD16a+ peripheral blood monocytes has been shown to correlate with degree of hypercholesterolemia and is dramatically reduced with lipid lowering therapy.[29] This phenotypic expression, in contrast to other phenotypes of monocytes, is shown to express high levels of inflammatory cytokines such as TNF-α whereas the anti-inflammatory IL-10 is low or absent. In addition, these cells are further characterized by an upregulation of cell surface adhesion molecules, suggesting an increased capacity for cell to cell interactions.[30]

The degree of macrophage infiltration has been shown to distinguish between unstable and stable coronary lesions. The preferential localization of macrophages in high-flow shoulder regions of the atherosclerotic plaque correlates with areas at highest risk for plaque instability. In contrast to controls, infiltrates of CD68-positive macrophages and CD3- and CD8-positive T-cells were statistically associated with the severity and frequency of superficial plaque inflammation and rupture.[31-33] This plaque instability, in part, stems from metalloproteinase (MMP-1 and MMP-2) production and release by activated macrophages within the inflamed atherosclerotic plaque.[34]

T-Lymphocyte

Antigen-presenting macrophages induced T-cell activation and results in inflammatory amplification through T-cell release of TNF-α, and INF-γ, further activating macrophages, platelets, and smooth muscle cells.[35] Levels of the main specific immune markers CD4+ and CD3+/DR+ T-cells, IL-2, and IgM have all been reported to be higher in unstable than in stable angina patients.[36] In addition, a higher percentage of IL-2 receptor positive T-lymphocytes in culprit lesions of patients with acute coronary syndromes indicate recent activation and amplification of the immune response within plaques. These

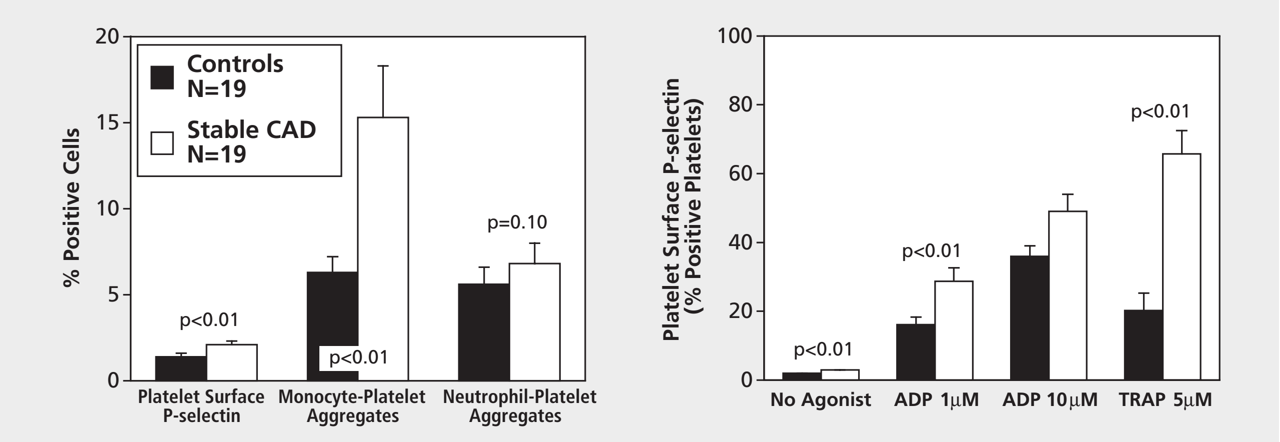

Figure 1. Augmented platelet activity and response to agonist in patients with stable coronary artery disease versus normal controls. (Reprinted with permission from the American College of Cardiology Foundation *Journal of the American College of Cardiology*, 1998, vol 31, pp 352-358.)

findings support the concept that a burst of inflammatory products could initiate or accelerate the onset of an acute coronary event.[37]

Mast cell

Mast cells have been recently identified to inhabit the vulnerable shoulder regions of the atherosclerotic plaque and to be associated with plaque erosion and rupture.[38,39] The population size of mast cell in athrectomy tissue correlates with the clinical severity of coronary syndromes. Their presence in the adventitia of ruptured plaques has led to the postulate that histamine release may provoke coronary spasm and contributes to the onset of myocardial infarction.[40,41] Mast cells have a primary role in the perpetuation of the inflammatory response in atherosclerosis, characterized by the production of TNF-α and neutral proteases (tryptase and chymase).[42,43] TNF-α stimulates macrophages and smooth muscle cells to produce two prometalloproteinases—prostomelysin and procollagenase. Subsequent activation of prometalloproteinases by mast cell produced tryptase and chymase leads to fibrous cap degradation and plaque destabilization.[44]

Neutrophil

As previously discussed, macrophages and T lymphocytes are the predominant cellular components of local inflammation within the atherosclerotic plaque. Neutrophils, although found sparsely in atherosclerotic plaques, play an integral part in the acute inflammatory response to tissue injury and have been implicated as a major factor in tissue damage in response to ischemia and reperfusion.[45] TNF-α, IL-8, IL-6, platelet-activating factor, and leukotrienes enhance neutrophil recruitment to ischemic and reperfused myocardium by augmenting cell adhesion molecule expression. The extent of accumulation has also been correlated to the degree of tissue injury.[46,47] A systemic activation of neutrophils has been reported in patients with angiographically documented coronary artery disease as compared with normal controls and a subset of trauma patients providing further proof for a chronic systemic inflammatory state in patients with atherosclerosis.[48]

Platelet

Traditionally, platelets have not been classified as inflammatory cells, but recent discoveries have led investigators to believe that platelets are critical constituents that tie in both inflammation and thrombosis. The presence of serologic markers of platelet activation is well established in the setting of an ACS.[49-51] Inflammatory cytokines induce the translocation of the cell adhesion molecule P-selectin to the surface of the platelet membrane, facilitating interactions among platelets, endothelial cells, and monocytes. Monocyte expression of tissue factor is induced by P-selectin and may be an initiator of thrombosis in areas of vascular injury.[52]

An initial step to answer the question of whether platelet activation is a result of or results in the development of an ACS was recently reported by Furman et al[53] In a flow cytometric analysis patients with stable coronary artery disease were shown to not only have increased levels of circulating activated platelets with enhanced P-selectin expression, but also to have an increased propensity to form monocyte-platelet aggregates **(Figure 1)**.[53] Additional evidence to implicate platelets as inflammatory mediators is the recent finding of their expression of CD40L. This transmembrane protein found on constituents of both cellular and humoral components of the inflammatory system is structurally related to TNF-α. CD40L is rapidly expressed by activated platelets and induces the expression of chemokines and cell adhesion molecules by endothelial cells thus provoking cell attraction, activation, and migration into the arterial wall.[54]

MARKERS AND MEDIATORS OF INFLAMMATION

C-Reactive protein (CRP)

Although many markers of inflammation have been associated with adverse cardiovascular outcome, CRP has been evaluated in every clinical phase of coronary disease. It therefore provides a superlative avenue to thoroughly discuss the prognostic significance of inflammatory markers in cardiovascular disease. CRP is an acute-phase reactant whose concentration in blood rises dramatically in response to nonspecific inflammatory stimuli. It has been convincingly linked to cardiovascular disease, initially in sera of patients after acute myocardial infarction and recently in the wall of human coronary arteries possibly linking its presence directly with the development of atherosclerosis.[55-57] Whether the association reflects a casual or direct interaction, elevated levels of CRP are associated with a worse prognosis in the full spectra of atherosclerotic disease.

In the setting of a Q-wave myocardial infarction, Anzai et al[58] reported that elevated levels of CRP were associated with cardiac rupture, left ventricular aneurysm formation, and 1-year cardiac death. Even though CRP was found to be an independent predictor of these events, there remained a confounding correlation to extent of cardiac enzyme elevation in those patients without revascularization procedures.[58] Therefore, CRP levels in this study may have reflected infarct size and subsequent risk for adverse outcome.

Tommasi et al[8] reported on the prognostic value of CRP levels in patients with a first acute myocardial infarction, uncomplicated in-hospital course, absence of residual ischemia, and normal left ventricular function. Only increased CRP levels were independently associated to the incidence of patients who developed cardiac events (cardiac death, new-onset angina, and recurrent myocardial infarction) **(Figure 2)**.[8] Importantly, there was no correlation between CRP levels and extent of rise of cardiac enzymes.

Although numerous studies have shown that an elevated CRP in the setting of unstable angina and non–Q-wave myocardial infarction is associated with worse prognosis,[6,7,59-61] Biassicci et al[62] reported on the prognostic significance of CRP elevation in patients with unstable angina without myocardial injury. They excluded those patients with elevated levels of cardiac enzymes at entry to avoid the interplay of myocardial necrosis on CRP and future events. They reported that an elevated discharge CRP was strongly associated with recurrent coronary instability and myocardial infarction **(Figure 3)** and, interestingly, 42% of patients had persistent elevation of CRP 3 months after hospital discharge. Adjunctive evidence that elevated CRP levels possess predictive power exceeding their association with myonecrosis is their independent and additive prognostic value to markers of myocardial injury, such as troponin T and I.[63,64]

In the Thrombolysis in Myocardial Infarction (TIMI) IIA trial, a dose-ranging trial for enoxaparin in UA and NQMI, elevated CRP correlated with increased 14-day mortality **(Figure 4)**. Most importantly, these findings existed even in patients with a negative rapid troponin T assay, thereby dissociating myonecrosis from CRP's prognostic power.[64] Milazzo et al[65] reported that in patients undergoing CABG a preoperative elevation of CRP has prognostic significance **(Figure 5)**. CRP levels <3 mg/L and ≥3 mg/L were associated with new ischemic events in 4% vs. 25% of patients, respectively.

In the setting of percutaneous coronary revascularization, a hyperresponsive reaction of the inflammatory system, defined by elevation of CRP, IL-6, and serum amyloid A after angioplasty, was recently presumed to portend a worse prognosis.[66] Gaspardone et al[67] confirmed this by showing a persistent elevation in CRP 72 hours after coronary artery stenting (excluding patients with periprocedural myocardial infarction) pinpointed all patients who later suffered an adverse outcome. In contrast, no cardiac events occurred in those with normal levels at 1 year follow-up **(Figure 6)**.

Ex vivo studies have recently introduced the concept that detecting heat release by inflammatory cells within an atherosclerotic plaque may predict future instability and rupture.[68] Stefanadis et al,[69] using a thermography catheter, demonstrated heterogeneity in heat production of 20%, 40%, and 67% in atherosclerotic plaques of patients with stable angina, unstable angina, and acute myocardial infarction, respectively. Most importantly there was a significant correlation between thermal heterogeneity and baseline CRP **(Figure 7)**.[69]

More conclusive evidence that chronic, indolent inflammation plays a principal role in the development and progression of atherosclerosis has come from the long-term follow-up of patients with no known atherosclerotic disease but increased levels of CRP. Among 14,916 apparently healthy men participating in the Physician's Health Study an elevated level of high-sensitivity CRP (HsCRP), which detects CRP levels as low as 0.175 mg/L, added to the predictive value of elevated lipids in predicting first myocardial infarction **(Figure 8)**.[70] Similarly, in the Women's Health Study, those who developed cardiovascular events had higher baseline CRP levels than control subjects, with the highest levels at baseline being associated with a five- and seven-fold increase in any vascular event and combined stroke or myocardial infarction, respectively.[71]

Additional evidence that CRP levels are strong predictors of future cardiac events in apparently healthy men was recently published from the Monitoring Trends and Determinants in Cardiovascular Disease Study (MONICA). Patients in the highest quintile of CRP level had a 2.6-fold increased risk of suffering a fatal or nonfatal myocardial infarction or sudden cardiac death.[72] These findings strongly support the pivotal role that inflammation plays in the destabilization of atherosclerosis.

The question remains what if any direct role CRP plays in the development of atherosclerosis. A possible explanation supporting CRP as an indirect cardiovascular risk factor is that it reflects inflammation related to coronary vessel pathogens, extent of atherosclerosis, myocardial necrosis, myocardial ischemia, and activity of circulating proinflammatory cytokines.[73] Direct evidence for CRP's role in the pathogenesis of atherosclerosis is that its presence in the arterial wall predicts severity of atherosclerosis and that it is able to bind to damaged membranes and lipids, activate complement, and stimulate production of tissue factor from activated macrophages.[74-77] Irrespective of its pathologic role, there is overwhelming evidence that CRP, a sensitive marker of inflammation, is a powerful predictor of future cardiac events in patients with Q-wave

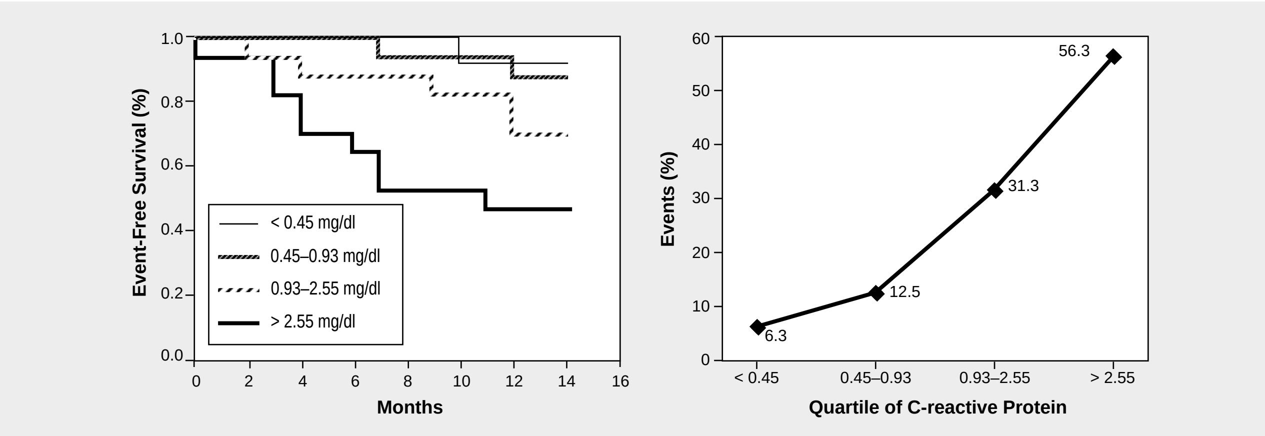

Figure 2. (Left) The event-free survival with respect to level of CRP in patients after an uncomplicated myocardial infarction. (Right) The distribution of events per quartile of CRP elevation. (Reprinted from the *American Journal of Cardiology*, vol 83, Tommasi et al, "C-reactive protein as marker for cardiac ischemic events in the year after a first, uncomplicated myocardial infarction," pp 1595-1599, Copyright 1999, with permission from Excerpta Medica.)

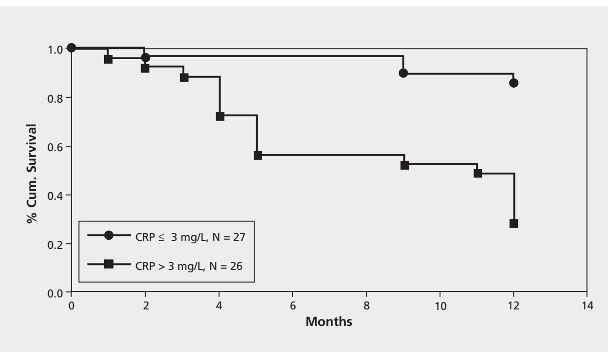

Figure 3. Cumulative event-free survival in patients with unstable angina, negative cardiac enzymes, and elevated discharge CRP. (Reprinted, with permission, from Biasucci et al, "Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability," Circulation 1999; 99(7):855-860.)

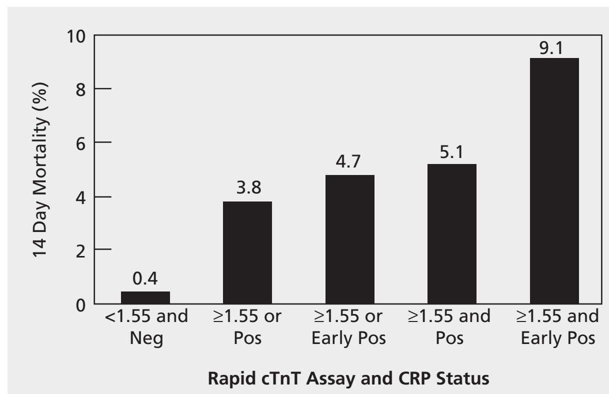

Figure 4. Independent and additive predictive value of CRP and cTNT (early positive defined by being positive in <10 minutes) on 14-day mortality. (Reprinted with permission from the American College of Cardiology Foundation *Journal of the American College of Cardiology*, 1998, vol 31, pp 1460-1465.)

MI, non-Q MI, unstable angina, stable angina, in patients who have undergone CABG and percutaneous coronary stenting, and recently in apparently healthy men and women. Recently the FDA has approved the use of a high-sensitivity CRP assay (Behring) as a prognostic test in the evaluation of patients with or expected atherosclerosis. This test has been most studied in those patients without clinically apparent atherosclerosis as a predictor of future cardiovascular events based on tertile of elevation.

Tumor necrosis factor-α (TNF-α) and other mediators

TNF-α is a pleiotropic proinflammatory cytokine with a wide range of effects that extend across a spectrum of pathologic conditions. Present in atherosclerotic lesions,[78] TNF-α appears to be one of the most important influences on the progression of atherosclerosis. Its upregulation is known to mediate and amplify a multitude of interactions resulting in progressive inflammation, plaque destabilization, and prothrombotic tendencies[79-87] **(Table 2)**. Treatment with a chimeric mAb to TNF-α has been shown to suppress inflammation and improve patient well-being in rheumatoid arthritis. Administration of anti-TNF-α Ab was recently shown to rapidly downregulate a spectrum of cytokines (IL-6), cytokine inhibitors (TNF receptors p75 and p55), and acute-phase proteins (amyloid A, haptoglobin, and fibrinogen).[88] This potent suppression of markers and mediators of inflammation may have tremendous potential in preventing progression of atherosclerosis.

IL-6 and IL-1 Ra (IL-1 receptor antagonist) not only have been shown to be elevated in the setting of ACS, but also are associated with increased risk of in-hospital events.[89] IL-6, produced by a variety of inflammatory cell types, has been shown to remain elevated up to 4 weeks after a myocardial infarction. Its properties increase fib-

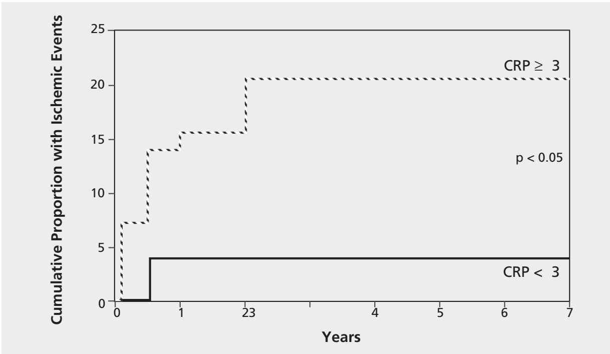

Figure 5. Cumulative proportion of ischemic events in patients with elevated CRP prior to coronary artery bypass grafting. (Reprinted from the *American Journal of Cardiology*, vol 84, Milazzo et al, "Elevated levels of C-reactive protein before coronary artery bypass grafting predict recurrence of ischemic events," pp 459-461, Copyright 1999, with permission from Excerpta Medica.)

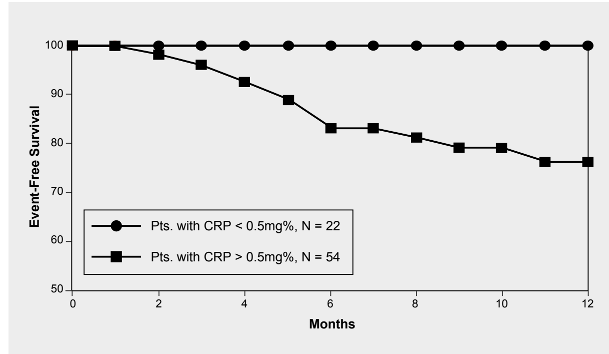

Figure 6. Event-free survival with respect to persistent elevation of CRP >72 hours after coronary stenting. (Reprinted from the *American Journal of Cardiology*, vol 82, Gaspardone et al, "Predictive value of C-reactive protein after successful coronary-artery stenting in patients with stable angina," pp 515-518, Copyright 1998, with permission from Excerpta Medica.)

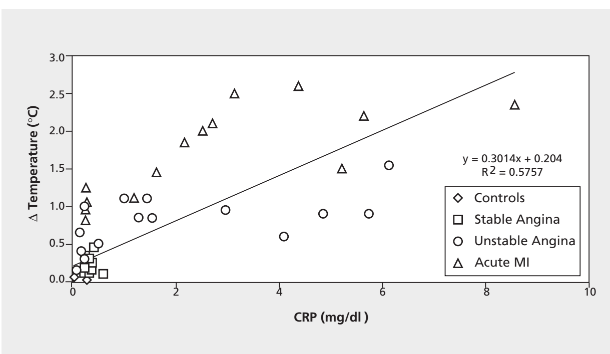

Figure 7. The correlation between thermal heterogeneity and level of CRP in control, stable angina, unstable angina, and acute myocardial infarction patients. (Reprinted, with permission, from Stefanadis et al, "Thermal heterogeneity within human atherosclerotic coronary arteries detected in vivo," Circulation 1999; 99(15):1965-1971.)

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Figure 8. Predictive value for lipoprotein(a), total homocysteine, total cholesterol, fibrinogen, t-PA antigen, ratio of total cholesterol to HDL, CRP, and CRP plus total cholesterol HDL ratio. (From Ridker PM et al, Ann Intern Med 1999; 130:933-937, with permission.)

rinogen and PAI-1, promote adhesion of neutrophils and myocytes during myocardial reperfusion, and produce a negative inotropic effect on the myocardium.[90-94] Pannitteri et al[95] reported that levels of IL-8 not only are elevated in the setting of acute myocardial infarction but that they precede the levels of IL-6 and parallel the kinetics of CPK. IL-8 is a powerful trigger for firm adhesion of monocytes to vascular endothelium, may play a potential atherogenic role by inhibiting local inhibitors of metalloproteinases in atherosclerotic plaques, and stimulates smooth muscle cell migration.[96,97] IL-4 and IL-13 have been shown to enhance the ability of activated human monocytes to oxidize LDL, thus potentiating its toxic effects.[98] OxLDL induces IFN-γ production by T-helper-1–like cells, which are known to inhibit local collagen synthesis by SMC, stimulate expression of tissue factor and CD40, and selectively induce MCP-1.[98-100] Many other cytokines have been implicated in immunity, inflammation, thrombosis, and angiogenesis.[101]

The above discussion underscores the vast trafficking, redundancy, and interplay of the cytokine system. Each mediator, though, must work through specific receptors and ultimately regulate gene expression of proteins vital to the potentiation and regulation of the inflammatory cascade. Nuclear factor κ-B (NF-κB), peroxisome proliferator-activated receptor activators (PPARs), CD40 receptor and its-ligand, and inducible cyclooxygenase enzyme (Cox-2) are avidly being investigated as we attempt to discover the final common pathway of inflammation and its role in atherosclerosis.

NF-κB

NF-κB is a transcription factor located in the cytoplasm of many cells as an inactive complex associated with a specific class of inhibitory proteins, called IκB. This complex binds and prevents nuclear translocation and DNA binding of NF-κB.[102] In response to inflammatory stimuli IκB is eventually degraded and NF-κB is released and transported to the nucleus. In the nuclei, NF-κB can initiate or regulate early response gene transcription by binding to promotor or enhancer regions.[103] NF-

TABLE 2
PROINFLAMMATORY AND THROMBOTIC PROPERTIES OF TNF-α

Inflammatory properties
- Regulation of macrophage colony-stimulating factor
- Regulation of cell adhesion molecules
- Modulation of smooth muscle cell phenotype
- Induction of IL-1 mRNA
- Inhibition of endothelial cell apoptosis

Plaque destabilization
- Induces smooth muscle cell interstitial collagenases
- Neutral effect on tissue inhibitors of metalloproteinases

Thrombotic properties
- Augments transcription and expression of tissue factor
- Decreases in activity of thrombomodulin-C and tissue-type plasminogen activator
- Increases production of plasminogen activator inhibitor
- Increases release of Von Willebrand factor

κB is known to regulate or be regulated by genes involved in every aspect of the proinflammatory cascade.[104,105] TNF-α and IL-1 are two important inducers, contributing to a positive feedback loop for NF-κB activation. As a consequence, there is a continuous upregulation of cytokines and perpetuation of inflammation.[103] NF-κB has been implicated in a variety of inflammatory diseases, such as allograft rejection, rheumatoid arthritis (RA), asthma, and inflammatory bowel disease.[104] In RA, NF-κB is overly expressed in synovial tissue, associated with surface expression of cell adhesion molecules, production of cytokines, and upregulation of the inducible isoform of cyclo-oxygenase (Cox-2). These processes are parallel to those found in atherosclerotic lesions.[104]

NF-κB activity is enhanced by known cardiac risk factors such as very low-density lipoprotein, OxLDL, hyperglycemia, and elevated levels of angiotensin II. On the contrary, its activity is inhibited by HMG-CoA reductase inhibitors, antioxidants, and gallates (phenolic compounds found abundantly in red wine).[106-111] Recently, Ritchie[112] reported data showing that NF-κB is activated in patients with unstable angina without evidence of myonecrosis and is therefore potentially linked in plaque disruption. Immunosuppression with glucocorticoids, gold, cyclosporin, FK506, and, importantly, aspirin and salicylates is known to inhibit NF-κB. Kopp et al[113] demonstrated that aspirin inhibits NF-κB activity by preventing the degradation of IκB, while Weber et al[114] established aspirin's ability to inhibit TNF-α–stimulated NF-κB activity.

CD40 and CD40L

CD40 is a phosphorylated 49-kDa glycoprotein expressed on B-lymphocytes, fibroblasts, monocytes, platelets, epithelial cells, and endothelial cells.[115] CD40L, also named CD154 or gp39, belongs to the TNF family of cytokines. The presence of CD40 and CD40L has been found in human atheroma, and their association is implicated with expression of cell adhesion molecules, cytokines, matrix metalloproteinases, and tissue factor.[54,115] Anti-CD40L has been shown to regulate autoimmune diseases such as lupus nephritis, skin and cardiac allograft rejection, and multiple sclerosis in experimental models.[116-118] Mach et al[119] reported a reduction in aortic atherosclerotic lesion size, fewer T-lymphocytes and macrophages, and a decreased presence of cell adhesion molecules in atheroma in cholesterol-fed mice lacking the LDL receptor when treated with anti-CD40L antibody.[119] Aukrust et al[120] recently reported elevated levels of CD40-CD40L in patients with angina pectoris. Patients with unstable angina had significantly higher levels than those with stable angina, allowing the authors to conclude that presence of CD40-CD40L may have a pathologic role in plaque destabilization and the development of ACS[120] **(Figure 9)**.

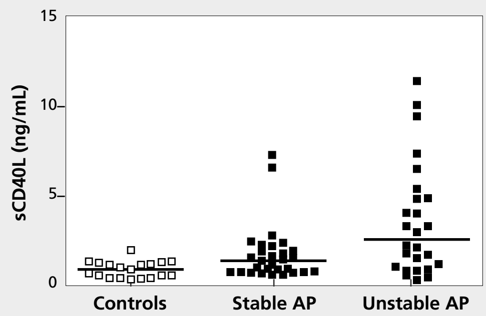

Figure 9. Levels of soluble CD40L in normal controls, stable angina, and unstable angina patients. (Reprinted, with permission, from Aukrust et al, "Enhanced levels of soluble and membrane-bound CD40 ligand in patients with unstable angina," Circulation 1999; 100(6):614-620.)

PPAR

Peroxisomal proliferator-activated receptors (PPARs), including PPAR-α, PPAR-γ, and PPAR-δ, are a group of nuclear transcription factors playing a key role in adipogenesis and lipid metabolism.[121] Recently, modulation of the development and progression of atherosclerosis has been substantiated by research that appears to link PPAR activity with the regulation of inflammation and plaque stability by their interactions with macrophages, endothelial cells, smooth muscle cells, and metalloproteinases. Ricote et al[122] found PPAR-γ to be upregulated in activated macrophages and to inhibit gelatinase B, nitric oxide synthase, and scavenger receptors. OxLDL has been shown to induce PPAR-γ expression in macrophages, resulting in monocyte differentiation and enhanced uptake in OxLDL.[123,124] Max et al[125] recently reported elevated levels of PPAR-γ expression on monocytes in human atherosclerotic lesions as compared to normal controls. Furthermore, PPAR-γ stimulation leads to a concentration-dependent decrement in monocyte-derived metalloproteinase activity. Finally, PPAR-α and -γ have been implicated in the induction of macrophage apoptosis through

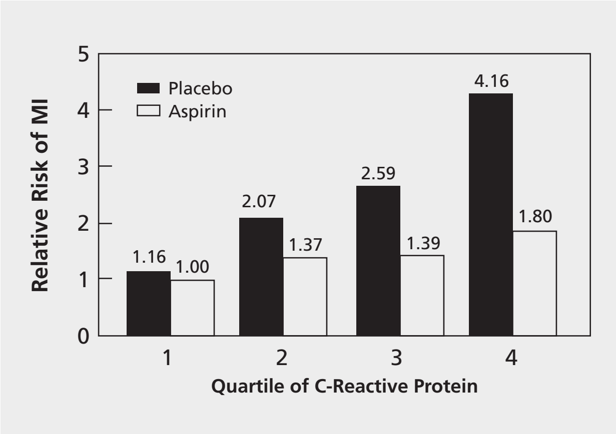

Figure 10. Relative benefit of ASA with respect to quartile of CRP. Data are shown allocated to ASA (open bars) and placebo (solid bars). (From Ridker et al, "Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men," N Engl J Med 1997; 336:973-979, with permission. Copyright © 1997 Massachusetts Medical Society. All rights reserved.)

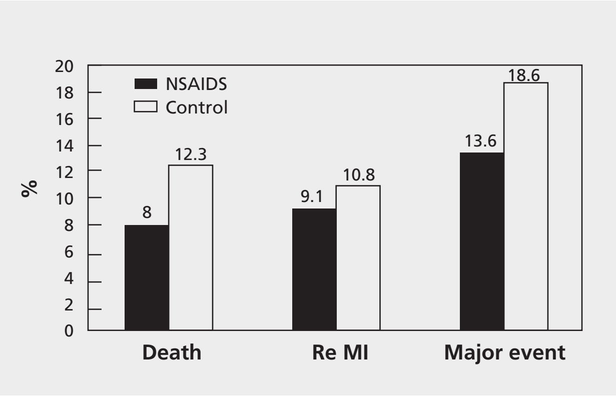

Figure 11. Cardiovascular events allocated to background of NSAID (solid bars) and control (open bars).

inhibition of NF-κB antiapoptotic pathways.[126] Endothelial cells also appear to be under the influence of PPARs by the regulation of leukocyte/endothelial cell interactions. Jackson et al[127] demonstrated an inhibitory effect of stimulated PPAR on endothelial cell expression of VCAM-1. In addition, stimulated PPAR-α has been shown to inhibit TNF-α-mediated endothelial cell VCAM-1 expression, COX-2 expression, IL-1 induced production of IL-6, and thrombin-induced endothelial-1 production.[128-130]

Key stimulatory PPAR ligands are naturally occurring prostaglandins, as well as synthetic antidiabetic and antilipidemic drugs. Gemfibrozil, a finofibrate and stimulator of PPAR-α, has recently been shown to dramatically reduce IL-1-induced production of IL-6, expression of COX-2 in human smooth muscle cells, and cardiovascular events in patients with low HDL levels. Importantly, this reduction in cardiovascular events was independent of LDL levels.[130,131] Troglitazone, an insulin sensitizer and PPAR-γ ligand, demonstrates a range of anti-inflammatory and potential plaque-stabilizing activities such as PPAR-γ–induced inhibition of macrophage metalloproteinases.[125]

Currently, the complex activities of PPARs and their ligands are not completely understood, although ligands with positive effects on lipid lowering (finofibrates) and glycemic control (troglitazone) would suggest that these transcriptional factors are clinically beneficial and mainly antiatherogenic.

Cyclo-oxygenase-2 (COX-2)

There are two distinct isoforms of cyclo-oxygenase (COX-1 and COX-2). These enzymes are necessary in the conversion of arachidonic acid to prostaglandin G2 and H2, which are potent agonists to the inflammatory cascade.[132,133] The ability of ASA and other NSAIDs to inhibit inflammation through their regulation of COX-1 was first described by Vane in 1971.[132] It was not until 1991 that an inducible form of the COX enzyme was discovered, COX-2.[134] Although a weak COX-2 inhibitor, aspirin and most available NSAIDs by virtue of their preferential COX-1 inhibitory effects, provide minimal anti-inflammatory action at doses not associated with significant side effects. COX-2 receptors are scantly expressed in the gastrointestinal tract or platelets and therefore likely provide augmented inflammatory control with few adverse effects.[135] COX-2 is felt to be the principal isoform that participates in inflammation and has been recently found to be widely expressed in atherosclerotic tissue.[136,137] Macrophage COX-2 mRNA expression has been shown to be induced by inflammatory cytokines such as INF-γ, TNF-α, and lipopolysaccharide, while other cytokines with anti-inflammatory properties, such as IL-10, have been shown to inhibit its induction.[138,139]

Speir et al[140] recently demonstrated a reduction in reactive oxygen species generation in CMV infected smooth muscle cells when pretreated with NSAIDs. This reduction was thought mainly to be due to inhibition of the COX-2 enzyme.[140] Although most investigations have described COX-2 as a proinflammatory mediator, recent reports by Cockerill et al[141] and Bishop-Bailey et al[142] have provided evidence for its anti-inflammatory potential. They demonstrated that HDL enhanced the expression of COX-2–dependent prostaglandin-I2, which is known to inhibit platelet and leukocyte activity. In addition, inhibition of IL-1-β resulted in upregulation of COX-2 and downregulation of the cell adhesion molecule ICAM-1. Although questions still remain, anti-inflammatory treatment such as aspirin, with its unquestionable beneficial effects, and a recent retrospective analysis of NSAIDs in patients after myocardial infarction that demonstrated a reduction in cardiac mortality and adverse events **(Figure 10)**,[143] suggest the potential for augmented clinical benefit with more potent and selective cyclooxygenase inhibition.

THE FUTURE OF INFLAMMATION CONTROL IN ACS

Aspirin, initially thought of mainly as an antiplatelet drug in the battle with atherosclerotic heart disease, is becoming more recognized for its anti-inflammatory properties. In addition to aspirin's COX-1 and weak COX-2 activity,

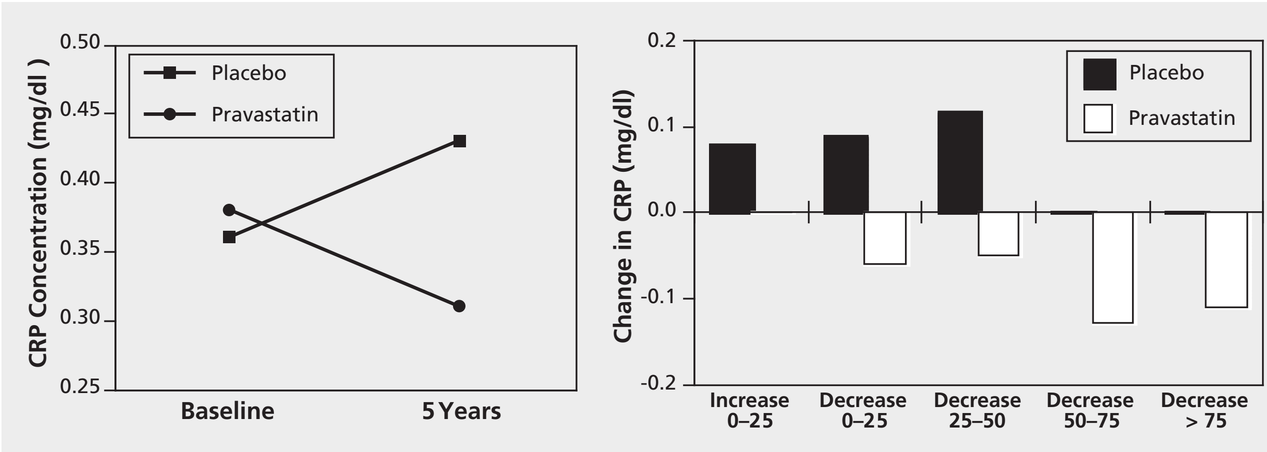

Figure 12. (Left) CRP at baseline and 5 years for patients treated with pravastatin or placebo. (Right) The change in CRP according to LDL level. Data are shown allocated to pravastatin (open bars) and placebo (solid bars). (Reprinted, with permission, from Ridker et al, "Long-term effects of pravastatin on plasma concentration of C-reactive protein," Circulation 1999; 100(3):230-235.)

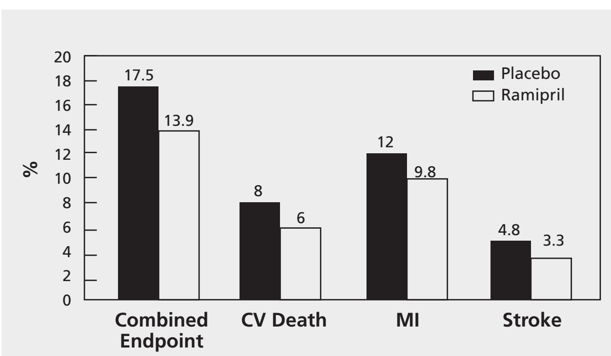

Figure 13. Cardiovascular endpoints allocated by patients receiving ramipril (open bars) or placebo (solid bars).

the inhibition of NF-κB activity is achieved by inhibiting both the degradation of IκB and effects of TNF-α. Clinical evidence to support aspirin's anti-inflammatory role has been reported by Ridker et al.[144] Aspirin reduced first MI in the Physicians Health Study, and this effect was directly related to the baseline CRP level[144] **(Figure 11)**. In addition, the recent negative results of the oral IIb/IIIa receptor inhibitor may be explained in part by the lack of aspirin's anti-inflammatory properties in the group receiving sole oral IIb/IIIa treatment.

A paradigm shift in thought may be evolving in favor of the anti-inflammatory properties of aspirin being more salient than its relatively weak antiplatelet effects in the reduction of ischemic cardiac events. HMG-CoA reductase inhibitors have been shown to dramatically reduce cardiovascular mortality and morbidity although the reduction in events is not linear with the reduction of LDL cholesterol below 125 mg/dL.[145] In an analysis of the Cholesterol and Recurrent Events (CARE) trial, Ridker et al[146] reported a significant 22% drop in CRP over a 5-year period in those treated with pravastatin versus placebo. Interestingly, CRP rose even in the placebo-treated arm which realized a reduction in LDL cholesterol **(Figure 12)**. Evidence continues to mount suggesting an anti-inflammatory role for HMG-CoA reductase inhibitors as these agents have been shown to alter regulation of DNA transcription, regulate natural-killer-cell cytotoxicity, inhibit platelet-derived growth factor-induced DNA synthesis, and decrease macrophage production of metalloproteinases.[146-149] ACE inhibitors have recently been demonstrated to possess potent anti-inflammatory properties that may explain their regulating effects on atherosclerotic driven endpoints. ACE inhibitors have been shown to exhibit antiproliferative and antimigratory effects on SMC and leukocytes, restore endothelial function, modulate platelet effects, and promote endogenous fibrinolysis.[150] The Heart Outcomes Prevention Evaluation (HOPE) study, a study of patients with vascular disease and no known heart failure, reported a dramatic and significant decrease in cardiovascular death, MI, and stroke in patients treated with ramipril versus placebo **(Figure 13)**.[151] Finofibrates and insulin sensitizers such as troglitazone are stimulators of PPAR receptors and are currently receiving attention for their anti-inflammatory and antiatherogenic potential. Unfortunately, not all methods of inflammatory control have realized a positive clinical outcome. Prevention of reperfusion injury in patients presenting with ACS by inhibiting leukocyte adhesion was recently reported from the HALT MI study. There was no significant reduction in infarct size and unfortunately a significant increase in infection rates in those randomized to high dose of the CD11/CD18 inhibitor.[152] This trial underscores the careful balance needed between adequate anti-inflammatory control and clinically significant immunosuppression.

Even though CRP and HsCRP have been shown to predict risk of future adverse cardiovascular events in virtually all patient subgroups, treatment options are limited to drugs not specifically heralded for their anti-inflammatory properties. Novel downstream approaches with the use of TNF-α, CD40L, NF-κB, and COX-2 inhibitors are under in vitro and animal investigations to determine their potential role in the battle against atherosclerosis. Treatment of atherosclerosis as an inflammatory disease should first focus on those pathogens known to initiate and propagate this disease, such as hypercholesterolemia, hypertension, diabetes, hyperhomocysteinemia, smoking, and possible infection. The second approach should be to uncover the etiology of the nearly 50% of patients who present with an ACS without known cardiac risk factors. Finally, further investigation is needed to determine the clinical efficacy of adjunctive anti-inflammatory therapy on the background of pathogen directed treatment.

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