Inflammatory Biomarkers in Stable Atherosclerosis

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Inflammation is a key mechanism in atherosclerotic progression and destabilization that precedes clinical events such as myocardial infarction. The inflammatory biomarkers provide a window into many of these inflammatory processes. In research studies, average levels of these biomarkers in groups of subjects are often related to the risk of clinical events, and modification of risk factors can change the plasma concentrations of many biomarkers, reflecting suppression of inflammation. More evidence exists for C-reactive protein (CRP) than for other inflammatory biomarkers, and the discussion of the clinical value of plasma levels of these markers is focused mainly on CRP. Although the inflammatory biomarkers are useful research tools, their value as a clinical instrument for assessment of cardiovascular risk and/or treatment efficacy is still widely debated. Each biomarker has passionate supporters who advocate these applications, but, at this stage, no inflammatory biomarker has universal support for clinical use and their potential for fulfilling this role requires further study. **(Am J Cardiol 2006; 98[suppl]:2P–8P)**

Once considered a passive process of lipid accumulation, atherosclerosis is now widely accepted as an active process of vascular cell activation, inflammation, and thrombosis.[1] Clinical events generally arise from disruption of the atherosclerotic intima due to changes related to all of these cellular events.[2,3]

Inflammation is a key process in the destabilization of atherosclerosis, and basic research, animal studies, and pathologic studies reveal a clear sequence of events that ultimately lead to clinical events. Inflammation is exacerbated by all of the cardiovascular risk factors identified in epidemiologic studies, and particularly by elevated low-density lipoprotein (LDL) cholesterol.[1] Clinical studies increasingly indicate that risk factor modification, the basis of preventing atherosclerotic events, also decreases inflammation in atherosclerosis.

However, inflammation in atherosclerosis is difficult to track directly. There are no imaging techniques that can monitor changes in inflammation, and arterial biopsy is not a practical or ethical way to study inflammation processes or to assess therapeutic interventions. It is in this setting that biomarkers of inflammation, typically measured from blood samples, are of such great interest.

The Explosion of Inflammatory Biomarkers

Biomarkers can provide useful information on inflammatory processes in research studies from blood or plasma samples that are collected prospectively or stored for many years in a frozen state. These markers provide a window into many of the processes involved with vascular cell activation, inflammatory cell recruitment, and activation of other cells in plaque (Figure 1).[4]

The inflammatory markers are potentially produced directly by inflammatory and vascular cells in plaque or indirectly in other important organs such as the liver. Of course, inflammation is a nonspecific event, and inflammation in other tissues due to other causes (eg, a pulmonary infection) generates waves of inflammatory biomarkers that can confound the relation to the more indolent inflammation that occurs in stable atherosclerosis syndromes.

The development of biomarkers of inflammation complements studies of markers of tissue damage (eg, troponin), markers of thrombosis and thrombolysis (eg, tissue plasminogen activator), and markers of lipid oxidation (eg, oxidized LDL) that are discussed elsewhere. Together, these biomarkers are able to provide insights into several important atherosclerotic processes in groups of subjects in research studies. However, their value in individuals, and ultimately in clinical practice, still requires refinement owing to a number of factors that include their biologic variability, the lack of standardization of some assays, and uncertainty regarding the added value from their use. For this reason, although each marker may have a group of

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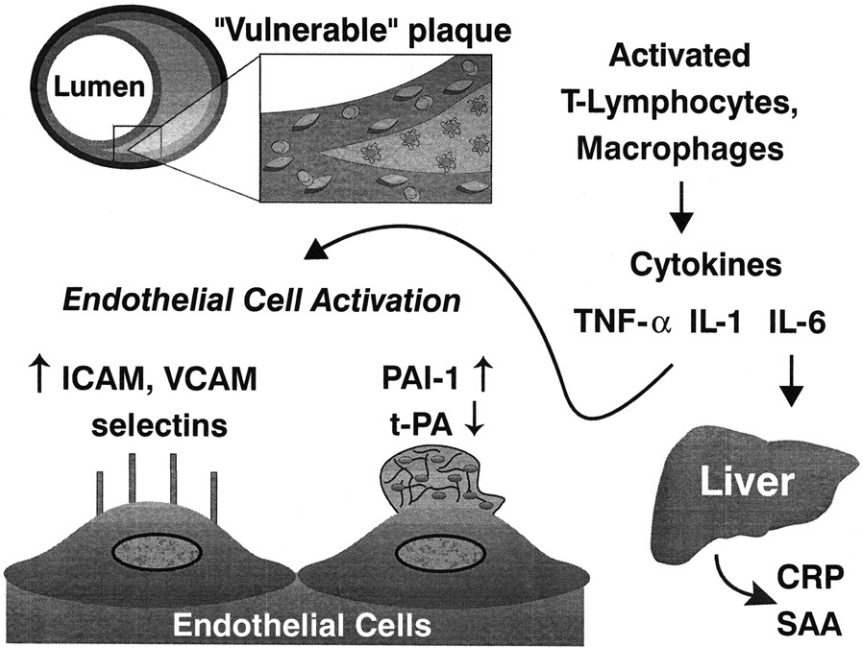

Figure 1. Inflammatory markers shed into the bloodstream provide a window into inflammatory processes in atherosclerosis. Cytokines expressed in cells within the vulnerable plaque such as interleukin (IL)-1, IL-6, and tumor necrosis factor–α (TNF-α) can induce synthesis and release of acute phase proteins such as C-reactive protein (CRP) and serum amyloid A (SAA) by the liver, or increase the expression of cellular adhesion molecules on endothelial cells (eg, intercellular adhesion molecule [ICAM], vascular cell adhesion molecule [VCAM], selectins) and potentially modulate prothrombotic factors (eg, plasminogen activator inhibitor (PAI) –1, tissue plasminogen activator [t-PA]). (Reprinted with permission from *Am J Cardiol*.[4])

passionate advocates, their use in clinical practice is still strongly debated.

Although the list of inflammatory biomarkers is extensive, the range of markers discussed below serves to illustrate the importance and excitement of this field. The responses of these markers to risk factor modification, and particularly the effect of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), serve to bolster their role as markers that reflect the inflammatory processes important in atherosclerosis.

C-Reactive Protein, Serum Amyloid A, and Interleukin-6

C-reactive protein (CRP), serum amyloid A (SAA), and interleukin (IL)-6 form an inflammatory axis. IL-6 is synthesized predominately by activated lymphocytes[1] but also by other activated vascular cells, and it is released into the bloodstream in minute quantities. CRP and SAA are acute-phase plasma proteins that are synthesized in the liver particularly in response to IL-6.[5] Both CRP and SAA are released into the bloodstream in much higher concentrations, thus amplifying the inflammatory signal of IL-6 by >100-fold.

Although plasma concentrations of IL-6 are related to subsequent atherosclerotic events in some studies,[6] the relatively small signal and the large diurnal variation in release in healthy individuals makes this a more difficult marker to study. In contrast, CRP is released in much larger amounts and, with the newer high-sensitivity assays, does not exhibit a diurnal variation, making measurements easier to interpret. SAA shares some of these features of CRP, although associations of CRP with health and disease are usually stronger.[7]

In cell culture, both CRP and SAA promote a number of proinflammatory cellular effects, including monocyte recruitment, activation of complement, and stimulation of cellular adhesion molecule and cytokine expression.[8–10] SAA also is closely related to high-density lipoprotein (HDL) and may displace apolipoprotein A-I from HDL to inhibit cholesterol efflux and reverse cholesterol transport.[11] However, these direct actions in cell culture have been questioned in recent years owing to the difficulty of completely removing other proinflammatory factors from the culture, the lack of controls in some studies, and the high concentrations of CRP and SAA required to achieve these changes.[12]

In pathologic studies of human atherosclerosis, CRP is sometimes found in regions with high macrophage density within plaques.[13,14] Although this is supportive evidence for a direct role of CRP on plaque inflammation or local synthesis of CRP by activated vascular cells, the presence of CRP in plaque does not prove that it is a central player in plaque destabilization. Equally plausible explanations are that CRP may be an “innocent bystander” or may be passively brought into plaque by macrophages from other inflammatory sites.

The clinical studies of CRP, SAA, and IL-6 consistently show a relation between plasma concentrations of these markers and atherosclerotic clinical events. The recent flurry of interest in these markers is due, in part, to the development of high-sensitivity assays and rapid throughput analytic systems for the markers. The high-sensitivity assays, only available for the past 5–6 years, enable researchers to look within the "normal" range of the older assays to identify risks associated with the subtle increases in inflammation more typical of the "grumbling" process associated with stable atherosclerosis.

In relatively healthy subjects, or in those with stable atherosclerosis syndromes, elevated levels of CRP (>3 mg/L) are related to the presence of peripheral artery disease in men[15] and to the future risk of myocardial infarction (MI) or sudden cardiac death.[16,17] In acute coronary syndromes (ACS), plasma concentrations of CRP, SAA, and IL-6 increase 10- to 20-fold, particularly with tissue necrosis marked by elevated myocardial enzymes (see the article by Ray and colleagues[18] elsewhere in this supplement). Regression analyses in large prospective cohort studies suggest that the relative risk of an event associated with CRP may be independent of other risk factors such as LDL cholesterol.[19,20] Although the statistical independence of CRP from LDL cholesterol in predicting clinical events implicates the inflammatory process, it does not provide a basis for assessing its incremental value in clinical practice.

Studies of risk factor modification, such as lowering LDL cholesterol, also support the concept that inflammatory biomarkers provide insights into processes occurring in the artery wall. Numerous studies show that lowering LDL cholesterol with statins reduces the concentration of CRP and to a lesser extent SAA.[21–24] It is more difficult to show consistent effects on IL-6,[24] but this may be related to the greater difficulty in measuring this biomarker.

Although correlation analyses show no, or only weak, relations between changes in LDL cholesterol and changes in CRP,[25] the temporal relation of the decline in both values is remarkably similar (Figure 2).[26] This suggests that the relation between LDL cholesterol and inflammation is greater than that measured by correlations between plasma LDL cholesterol and CRP.

The clinical value of using these inflammatory biomarkers to assess cardiovascular risk and the success of therapy is widely debated. More evidence exists for high-sensitivity CRP than for other biomarkers and most discussion of the clinical use of biomarkers continues to focus on CRP.

Intercellular Adhesion Molecule–1 and Vascular Cell Adhesion Molecule

The cellular adhesion molecules, intercellular adhesion molecule (ICAM)–1 and vascular cell adhesion molecule (VCAM), are important in leukocyte trafficking, which initiates and promotes plaque inflammation.[27] Cellular adhesion molecules are expressed on the luminal surface of activated endothelial cells. In conjunction with the related selectins, they capture lymphocytes and monocytes from the circulation and facilitate their recruitment into the arterial intima.[27] The cellular adhesion molecules are also cleaved from the surface of the endothelial cell, and these shed components are released into the bloodstream.[28] In cell culture, the concentration of shed or soluble cellular adhesion molecules is related to the concentration of cellular adhesion molecules expressed on the endothelial surface.

Elevated plasma concentrations of cellular adhesion molecules are related to the carotid intimal thickness (a risk factor for clinical events)[29,30] and to the risk of atherosclerotic events in some, but not all, clinical studies.[15,31–33] Similarly, in some clinical studies, statin therapy lowers the plasma concentrations of these biomarkers.[34,35]

The soluble cellular adhesion molecules provide insights into mechanisms important in inflammatory cell recruitment. However, their relation to risk, the effects of risk factor modification, and the interpretation of changes are less well studied compared with CRP, and these issues require further elucidation.

Monocyte Chemoattractant Protein–1

Monocyte chemoattractant protein (MCP)–1 is a chemokine secreted by activated endothelial and smooth muscle cells in the artery wall. MCP-1 is among the many chemokines that help recruit lymphocytes and monocytes into the arterial intima and activate these cells to promote atherosclerosis.[36]

In animal studies of atherosclerosis, MCP-1 is widely expressed in plaque,[37] and inhibition of MCP-1 activity is associated with inhibition of neointimal hyperplasia,[38] reduced vascular medial thickening,[39] and reduced plaque formation.[40]

The relation of plasma concentrations of MCP-1 to atherosclerosis is less well known compared with other inflammatory biomarkers. Elevated MCP-1 levels have been reported in separate studies in patients with peripheral artery disease[41] compared with controls and in patients with an increased risk of restenosis after coronary angioplasty.[42] In the Dallas Heart Study, plasma levels of MCP-1 in 3,499 subjects were associated with traditional risk factors for atherosclerosis as well as with CRP and coronary artery calcification.[43]

Interleukin-1β

IL-1β is a proinflammatory cytokine released by immune cells.[44] The activity of this cytokine is tightly regulated by proteases required for activation, by a naturally occurring receptor antagonist (IL-1Ra), and by IL-1RII, a decoy receptor.[44,45]

Although studied less extensively than other inflamma-

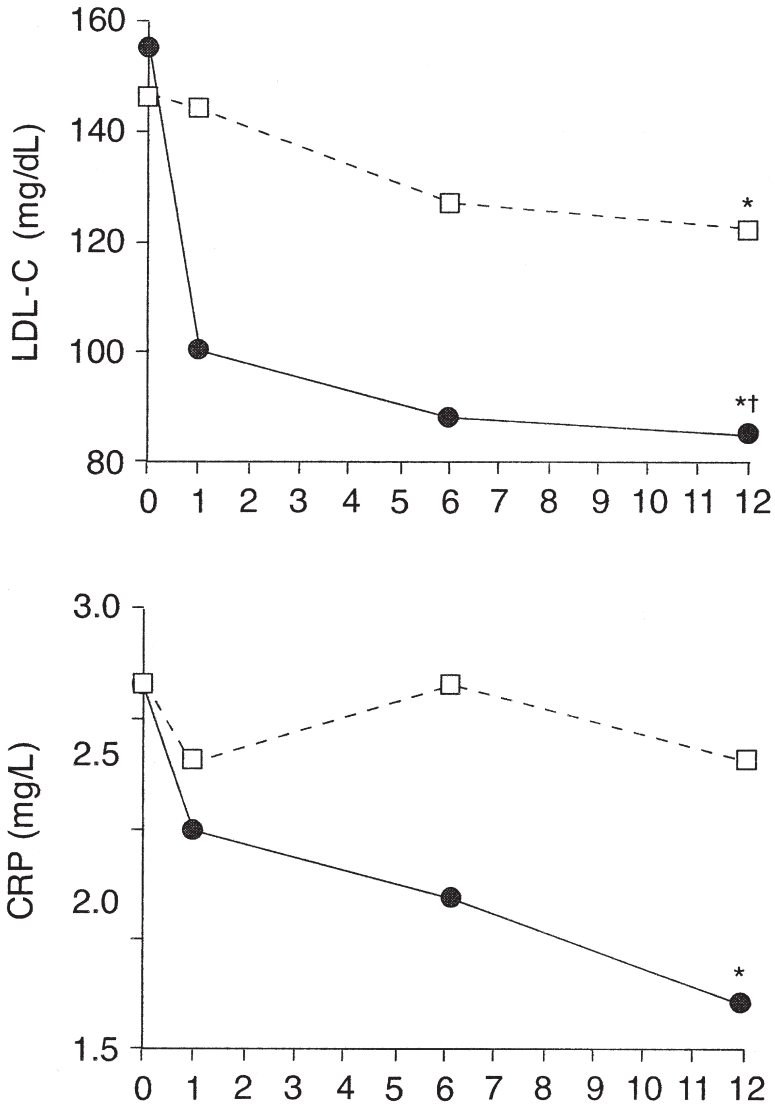

Figure 2. The temporal decrease in low-density lipoprotein cholesterol (LDL-C) with statin therapy closely parallels the decline in C-reactive protein (CRP). Solid curves represent levels of LDL-C (*top*) and CRP (*bottom*) in patients randomized to intensive LDL-C reduction to a goal of <80 mg/dL [1 mg/dL = 0.0259 mmol/L], and dotted curves represent levels in patients randomized to less intensive LDL-C lowering to a goal of <130 mg/dL. *p <0.01 vs baseline; †p <0.001 for changes with intensive versus moderate LDL-C lowering. (Reprinted with permission from *Am J Cardiol*.[26])

tory markers, plasma concentrations of IL-1β are elevated in patients with hyperlipidemia,[46] and statin treatment may lower IL-1β levels in patients with hypercholesterolemia.[34,47] However, regulation of IL-1β and its responses to statin treatment may be more complex than other markers. A substantial amount of this cytokine in the sera of patients with hypercholesterolemia may be derived from platelets.[47] Clinical studies are assessing the relations among IL-1β, its endogenous antagonists, and atherosclerotic clinical events.

Fas/Fas Ligand

Fas is a type I membrane protein of the tumor necrosis factor receptor superfamily that induces apoptosis on binding to the Fas ligand and activation of an intracellular cascade of proteases.[48] Fas is expressed in virtually all cell types, whereas the expression of Fas ligand is restricted to activated T lymphocytes, vascular endothelial cells, and immune-privileged tissues. Soluble Fas is generated by alternative messenger RNA splicing, whereas soluble Fas ligand is released in serum from membrane-bound Fas ligand processed by a metalloproteinase, and each of these may have opposing effects on apoptosis.

The Fas/Fas ligand system is involved in a wide variety of immunologic processes with various proinflammatory and anti-inflammatory effects. In addition, apoptotic smooth muscle cells in the intima express high levels of Fas, suggesting a role for the Fas/Fas ligand system in remodeling and plaque rupture.[49]

Relatively few studies have examined the relation of soluble Fas and soluble Fas ligand to atherosclerotic events.

In a study of patients with congestive heart failure, levels of soluble Fas, but not soluble Fas ligand, were associated with prognosis.[50] In another study, soluble Fas was associated with atherosclerotic events in patients with renal failure.[51,52]

The effect of statins on Fas and Fas ligand is uncertain at this time. In cell culture, atorvastatin and simvastatin reduce the expression of Fas ligand on human T cells,[53] suggesting that statins may reduce the cytotoxicity of activated T cells in plaque by this mechanism.

A small number of clinical studies have examined the effect of statins on soluble Fas/Fas ligand. In the Atorvastatin on Inflammatory Markers (AIM) study, plasma concentrations of soluble Fas were elevated in patients at high cardiovascular risk compared with healthy controls.[54] The presence of the metabolic syndrome was associated with higher levels of soluble Fas. Treatment with atorvastatin for 12 weeks lowered soluble Fas levels in the population as a whole as well as in patients with the metabolic syndrome.[54] In a separate study, patients with familial-combined hyperlipidemia or carotid atherosclerosis were found to have reduced levels of soluble Fas ligand, perhaps indicating endothelial dysfunction, but those levels were restored by treatment with atorvastatin for 1 year.[55] Future studies of soluble Fas and soluble Fas ligand may help elucidate the role of mechanisms that control vascular cell apoptosis and cytotoxicity in atherosclerotic plaque.

Debate on the Clinical Value of Inflammatory Biomarkers

Most of our evidence for the relation between inflammatory markers and cardiovascular disease, or prognosis and therapy, centers on CRP. The prognostic value of inflammatory markers depends on how much additional information they give beyond the conventional markers of risk. Their value for determining therapy depends on whether patients with an otherwise low level of risk, but with high levels of inflammatory markers, obtain benefits from drug therapy (given that lifestyle changes apply to everyone).

Although statins reduce CRP in patients with stable atherosclerosis, the value of measuring CRP in lower-risk individuals to determine who would benefit from statin therapy is still debated. This question will be inadequately addressed by the Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) study. This study is recruiting patients without coronary artery disease who have LDL cholesterol levels <130 mg/dL [1 mg/dL = 0.0259 mmol/L] and CRP concentrations >2 mg/L.[56] Approximately 15,000 subjects will be randomized to either rosuvastatin (20 mg/day) or placebo for 3 years to test the hypothesis that patients with low LDL cholesterol levels and high CRP levels will benefit from statin therapy. However, the selection of patients with LDL cholesterol levels <130 mg/dL and CRP levels >2 mg/L raises questions about whether the study is really examining low-risk patients. This combination is typical of patients with insulin resistance and the metabolic syndrome, and those with multiple risk factors. Arguably, many of these patients should be treated because of their elevated total or global risk, and a reduction in risk with statin therapy is highly likely (as in the Air Force/Texas Coronary Atherosclerosis Prevention Study [AFCAPS/TexCAPS]).[57]

A more informative study would be one that also randomized patients with a similar risk factor profile (ie, LDL cholesterol <130 mg/dL), but with CRP <2 mg/L, to statin or placebo. If these patients also had a reduction in risk with statin therapy, there would be no value in knowing CRP concentrations. Alternatively, if statin therapy did not reduce the risk of events in the latter group, this would support efforts to measure CRP in patients with this risk profile to select those eligible for statin therapy.

The uncertainty in using biomarkers clinically has led to the lukewarm reception by the authors of national guidelines. In 2003, the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) published evidence-based guidelines for inflammatory markers.[58] The only American College of Cardiology (ACC)/AHA Class I recommendation in this document was that CRP should be measured in milligrams per liter. The use of inflammatory biomarkers in clinical practice did not achieve an ACC/AHA Class I recommendation.

Conclusion

Inflammation plays a crucial role in promoting and destabilizing atherosclerotic plaque that subsequently leads to clinical syndromes in cardiovascular disease. The inflammatory biomarkers measured from easily obtained blood samples are providing a window on the relation of inflammatory mechanisms to disease and the effects of risk factor modification, particularly by use of statin therapy.

The evidence that links inflammatory markers to disease and prevention of disease is much greater for some inflammatory markers, such as CRP, than for others. These markers provide valuable tools to study disease progression and new prevention strategies. However, their value in clinical practice is still being investigated.

Clinical studies can augment the information from assessing clinical outcomes, by measuring some of these biomarkers in stored plasma specimens, and this is likely to provide further insights and support for preventive therapies. At this stage, most studies have provided mechanistic support for the value of many of our preventive therapies, in particular lipid lowering with the statin class of drugs.

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