Special Review

New Markers of Inflammation and Endothelial Cell Activation
Part I

Paul E. Szmitko, BSc; Chao-Hung Wang, MD; Richard D. Weisel, MD; John R. de Almeida, BSc; Todd J. Anderson, MD; Subodh Verma, MD, PhD

Current views regard atherosclerosis as a dynamic and progressive disease arising from the combination of endothelial dysfunction and inflammation.1–6 The vascular endothelium, located at the interface of blood and tissue, is able to sense changes in hemodynamic forces and bloodborne signals and react by synthesizing and releasing vasoactive substances. Vascular homeostasis is maintained by a balance between endothelium-derived relaxing and contracting factors. With disruption of this balance, mediated by inflammatory and traditional cardiovascular risk factors, the vasculature becomes susceptible to atheroma formation. Inflammatory mediators appear to play a fundamental role in the initiation, progression, and eventual rupture of atherosclerotic plaques. As evidence accumulates linking inflammatory processes to atherogenesis, markers of inflammation and endothelial activation may become useful by providing additional information about a patient’s risk of developing cardiovascular disease, as well as providing new targets for treatment.7,8 This review article is the first part of a two-article series examining emerging markers of inflammation and cardiovascular disease. Part I will provide a brief overview of the link between inflammation, endothelial dysfunction, and atherosclerosis and will begin highlighting emerging inflammatory mediators of endothelial cell (EC) activation, a discussion that will be continued in Part 2.

Endothelial Dysfunction: Setting the Stage for Inflammation

Endothelial dysfunction is a broad term that implies diminished production or availability of nitric oxide (NO) and/or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors, such as endothelin-1 (ET-1), angiotensin, and oxidants.1 NO, generated by the conversion of the amino acid l-arginine to NO and l-citrulline by the enzyme NO synthase, is the key endothelium-derived relaxant protein-1 (MCP-1), and upregulate vascular cell adhesion molecule-1 (VCAM-1) on ECs.14–16 Newer risk factors such as elevated C-reactive protein (CRP) levels can also promote endothelial dysfunction by quenching the production of NO and diminishing its bioactivity.17 These endothelial modifications promote inflammation within the vessel wall, setting the stage for the initiation and progression of an atherosclerotic lesion.

Initiation of Inflammation and Atherosclerosis

When ECs undergo inflammatory activation, the increased expression of selectins, VCAM-1, and intercellular adhesion molecule-1 (ICAM-1) promotes the adherence of monocytes. Adhesion molecule expression is induced by proinflammatory cytokines such as IL-1β and tumor necrosis factor-α (TNF-α), by the acute-phase protein CRP that is produced by the liver in response to IL-6, by protease-activated receptor signaling, by oxLDL uptake via oxLDL receptor-1 (LOX-1),
and by CD40/CD40 ligand (CD40L and CD154) interactions.18–22 Once adherent, the monocytes transmigrate into the tunica intima, the innermost layer of the arterial wall, passing between the ECs. This monocyte migration is directed along a concentration gradient of MCP-1, via interaction with the monocyte receptor CCR2.23 Once within the arterial intima, the monocytes develop into macrophages and begin to express scavenger receptors, such as SR-A, CD36, and LOX-1, that internalize modified lipoproteins.24,25 Internalization of these lipoprotein particles gives rise to lipid-laden macrophages or foam cells, which characterize early atherosclerotic lesions. Within the developing atheroma, the foam cells begin to secrete proinflammatory cytokines that maintain a chemotactic stimulus for adherent leukocytes, augment expression of scavenger receptors, and promote macrophage replication.6

However, macrophages are not alone in contributing to atheroma formation. T cells, dendritic cells, and mast cells are also recruited into atheromatous plaques.26 Binding to adhesion molecules, such as VCAM-1, facilitates T-cell entry into the intima. Once within the arterial intima, T cells may become activated by encountering antigens such as ox-LDL and begin to secrete cytokines that can influence macrophage activity. CD40/CD40L engagement between activated T cells and macrophages can result in the expression of tissue factor (TF), matrix metalloproteinases (MMPs), and proinflammatory cytokines that perpetuate the inflammatory response.6 Plaque formation is further promoted by the less abundant mast cells. On mast cell degranulation, TNF-α, heparin, and serine proteases are released.6 If the risk factors inducing endothelial dysfunction and inflammation remain, the atheroma will progress from a fatty streak to a more complex lesion.

Progression of Inflammation and Atherosclerosis
The evolution of a fatty streak toward a complex lesion is typified by the proliferation of smooth muscle cells (SMCs), their migration toward the intima, and their synthesis of collagen. Continued release of cytokines, such as MCP-1, by activated ECs, T cells, and foam cells not only perpetuates inflammation and lipid accumulation within the atheroma but also influences SMC activity.22,27 Expansion of this lesion within the coronary arteries can result in lumen obstruction, causing a reduction of blood flow, which may present clinically as angina. Neovascularization supports plaque growth, and rupture of these newly formed, fragile vessels is postulated to result in an acute expansion of the lesion.28 However, lipid core growth, whether progressive or acute, eventually causes destabilization of the plaque.

Proinflammatory cytokines secreted by activated T cells, such as interferon (IFN)-γ, can limit the synthesis of new collagen required for fibrous cap preservation.6 Accumulation of oxLDL has toxic effects on macrophages and SMCs, leading to necrotic core formation.29 Implicated with oxLDL toxicity is lipoprotein-associated phospholipase A₂, an enzyme that, when inhibited, reduces macrophage death.30 The death of macrophage foam cells leads to lipid spillage, promoting further inflammation, while SMC death further reduces collagen synthesis and promotes fibrous cap thinning. The thinning of the fibrous cap is enhanced by the overexpression of MMPs, interstitial collagenases, and gelatinases, which degrade supportive collagen.31 MMP overexpression and activation within the plaque are mediated by IL-1β, TNF-α, oxLDL, and CD40L. Once the fibrous cap is weakened, the plaque is vulnerable to rupture, precipitating acute thrombotic complications.

The Final Frontier: Vulnerable Plaque
Disruption of the vulnerable atherosclerotic plaque, on exposure to hemodynamic stresses, can trigger thrombosis, culminating in acute myocardial infarction. Erosion of the plaque surface, characterized by areas of EC desquamation, exposes a prothrombotic surface. An even greater prothrombotic stimulus arises from the rupture of a fibrous cap and the spilling of its contents into the lumen. Subendothelial collagen, TF, and von Willebrand factor become accessible to components in the circulation, promoting coagulation and thrombin formation.6 Platelet activation and aggregation ensue, mediated by interactions with thrombin, TF, and von Willebrand factor. TF overexpression by ECs and macrophages is enhanced by the presence of inflammatory mediators within the plaque, namely IL-1, TNF-α, and CD40L. In response to this vascular insult, thrombogenicity is further favored by the activation of protease-activated receptors on platelets and in the adjacent tissue.32 Thus, inflammation participates in all steps of atherosclerosis. As these inflammatory pathways are deciphered, new inflammatory mediators may emerge, providing clinicians with additional information regarding a patient’s risk for cardiovascular disease and new targets for therapeutic intervention.

Novel Inflammatory Mediators of Inflammation and EC Activation CRP
Amid the surge of inflammation research, one singular observation that has generated extraordinary interest is the acute-phase reactant CRP. Accumulating evidence suggests that circulating high-sensitivity CRP represents one of the strongest independent predictors of vascular death in a number of settings.33–35 Indeed, CRP appears to be a stronger predictor than LDL cholesterol, and it adds prognostic value to conventional Framingham risk assessment.36 The link between CRP and atherosclerosis was initially suggested to be that of a biomarker versus a mediator of atherosclerosis. This dogma has been recently revisited, with observations from our group and others suggesting that CRP has a direct effect to promote atherosclerotic processes and EC inflammation (Figure 1).17,20,37–41 Human recombinant CRP, at concentrations known to predict vascular disease, elicits a multitude of effects on endothelial biology favoring a proinflammatory and proatherosclerotic phenotype. For example, CRP potently downregulates endothelial NO synthase (eNOS) transcription in ECs and destabilizes eNOS mRNA, with resultant decreases in both basal and stimulated NO release.17 In a synchronous fashion, CRP has been shown to stimulate ET-1 and IL-6 release, upregulate adhesion molecules, and stimulate MCP-1 while facilitating macrophage LDL uptake.20 More recently, CRP has been shown to facilitate EC apoptosis and inhibit angiogenesis (S. Verma et
al, unpublished observations, 2003) while augmenting CD14-induced EC activation (R.P. Palusinski et al, unpublished observations, 2003). Preliminary observations from our group indicate that CRP also potently upregulates nuclear factor κB, a key nuclear factor facilitating transcription of numerous proatherosclerotic genes. The proatherosclerotic effects of CRP also appear to be modified by risk factors and treatment strategies. For example, hyperglycemia potentiates the effects of CRP on EC activation, and pharmacological interventions with statins, glitazones, and bosentan, an endothelin receptor antagonist, attenuate these processes. In addition to having direct effects to promote EC activation, CRP appears to function in a fashion that inhibits bone marrow–derived endothelial progenitor cell survival and differentiation.42 Endothelial progenitor cells have been suggested to play an important role in postnatal neovascularization, and the ability of CRP to inhibit progenitor cell survival may be an important mechanism inhibiting compensatory angiogenesis in chronic ischemia. Thus, CRP is not only an inflammatory marker of atherosclerosis/coronary events but is also a mediator of the disease because it contributes to the substrate underlying lesion formation, plaque rupture, and coronary thrombosis via interacting with, and altering, the EC phenotype.

The direct proatherogenic effects of CRP extend beyond the endothelium to the vascular smooth muscle. Recent evidence suggests that CRP, at concentrations known to predict cardiovascular events, directly upregulates angiotensin type 1 receptor in vascular SMCs in vitro and in vivo, and stimulates vascular smooth muscle migration, proliferation, neointimal formation and ROS production.35 Taken together, these data lend credence to the notion that CRP functions as a proatherosclerotic factor, in addition to a powerful risk marker.

**CD40/CD40L Signaling Dyad**

Originally identified in B and T lymphocytes as being involved in T-cell–dependent B-cell activation and differentiation, the CD40/CD40L system has since been implicated in the pathophysiology of severe chronic inflammatory diseases, including atherosclerosis.22,43,44 CD40, a 50-kDa integral membrane protein of the TNF receptor family, and CD40L, a 39-kDa member of the TNF family, are coexpressed by all of the major cellular players in atherosclerosis, namely activated T lymphocytes, vascular ECs, SMCs, and macrophages.22 Both the receptor and ligand are functional, mediating various proatherogenic processes. Immunohistochemistry studies revealed the presence of the CD40/CD40L signaling dyad within both early and advanced human atherosclerotic plaques.45,46 The importance for CD40 signaling in atherosclerotic plaque development and evolution was demonstrated using LDL receptor–deficient mice that were fed a high-cholesterol diet.57,48 By interrupting CD40 signaling in these mice, using a neutralizing anti-CD40L antibody, both the de novo formation and the further progression of established atherosclerotic lesions were drastically reduced. Further evidence suggesting a link between atherosclerosis and the inflammatory properties of CD40/CD40L emerges from studies examining circulating levels of soluble CD40L (sCD40L), which is primarily derived from activated platelets and is considered to possess biological activity.49 Elevated
plasma concentrations of sCD40L were observed in patients with unstable angina and predicted patients with features of high-risk atherosclerotic lesions as well as the risk for future cardiovascular events in women. A recent study examining sCD40L in acute coronary syndromes found that elevated sCD40L levels indicated a significantly increased risk of death or nonfatal myocardial infarction, a risk that was significantly reduced with abciximab. Also, a general upregulation of the CD40 system was observed in patients with moderate hypercholesterolemia, and elevated levels of sCD40L were reported in individuals with type 1 or 2 diabetes. The clinical associations of the CD40/CD40L proinflammatory system and atherosclerosis suggest that CD40 signaling function spans from early atherogenesis to late thrombotic complications.

The initial trigger for CD40/CD40L expression within the atheroma remains uncertain, but a recent study suggests that oxLDL may play this role. OxLDL induced the expression of CD40 and CD40L in human ECs, SMCs, and macrophages, induction that was diminished on statin administration. Endothelial dysfunction and the subsequent changes in blood flow promote CD40-mediated endothelial activation by decreasing the intracellular expression of a CD40 signaling blocker. CD40 signaling in ECs stimulates the production of ROS, which in turn antagonize endothelial NO production, which assists in the perpetuation of a dysfunctional endothelium. Endothelium activation and CD40/CD40L then work in concert to initiate atherosclerotic lesion formation. Ligation of CD40 on ECs and SMCs induces the expression of adhesion molecules such as E-selectin, VCAM-1, and ICAM-1, promoting the recruitment of monocytes and lymphocytes to the lesion. Leukocyte recruitment is further enhanced by CD40L-induced secretion of MCP-1, IL-1, IL-6, and TNF-α by the atheroma-associated cells. CD40L on activated platelets is able to trigger this inflammatory reaction in ECs, inducing ECs to secrete chemokines and to express adhesion molecules, events that promote the recruitment and extravasation of leukocytes at the site of injury. These cytokines and chemokines amplify the inflammatory effect and serve to foster a proatherogenic environment.

CD40L-induced pathways contribute to conditions that favor plaque progression toward instability. The balance between the synthesis and breakdown of collagen, the predominant structural component of the fibrous cap, is shifted toward degradation by CD40 ligation. With the ligation of CD40, human vascular ECs, SMCs, and macrophages increase expression of a full complement of MMPs, including the interstitial collagenases MMP-1, MMP-8, and MMP-13. Plaque stability and lesional collagen content were actually increased on interruption of CD40 signaling. In addition to making the plaque more fragile, CD40 signaling induces TF expression in both ECs and SMCs. Elevated levels of TF enhance the thrombogenic potential of the plaque on rupture by instigating the extrinsic pathway of blood coagu-
Furthermore, CD40 has recently been shown to be constitutively expressed on the surface of platelets and that its ligation results in platelet activation, which may serve to further enhance platelet-driven thrombus formation.63 Finally, CD40L inhibits EC migration. 58 By preventing ECs to migrate, reendothelialization of any plaque erosions is impaired, enhancing the possibility of an acute atherosclerotic event. Thus, the proinflammatory dyad, CD40/CD40L, participates in inducing not only proatherogenic but also prothrombotic conditions (Figure 2).

Interleukin-18
IL-18, a member of the IL-1 cytokine family, was originally identified in macrophages and Kupffer cells as a factor able to induce IFN-γ production by T cells.54 IFN-γ itself is a central proatherogenic factor as demonstrated by a significant reduction in atherosclerotic lesion development in hypercholesterolemic mice that lacked IFN-γ.65 IL-18 functions by signaling through its receptor that is expressed on the T-helper (Th) 1 subpopulation of lymphocytes, ECs, SMCs, and macrophages, all cellular components of the atheromatous plaque.56,67 Proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 induce IL-18 gene expression by human macrophages and in turn, ligation of the IL-18 receptor induces the expression of these same cytokines that have been implicated in atherogenesis.68,69 This creates a positive feedback loop that promotes a persistent inflammatory state that if occurring in the vasculature will not only promote the formation and maintenance of a plaque but will also contribute to its progression.

IL-18 is highly expressed in atherosclerotic plaques, as compared with normal arteries and is localized mainly in plaque macrophages.67,70 Significantly higher levels of IL-18 mRNA were found in unstable plaques, and serum levels of IL-18 were determined to be greater in patients who have suffered a myocardial infarction or who experience unstable angina.70–72 After adjustment for ejection fraction, IL-6, and CRP levels, serum IL-18 levels remained a strong independent predictor of death from cardiovascular causes in patients with coronary artery disease.71 Animal models support the proatherogenic role of IL-18 and the beneficial effect its inhibition has on plaque progression.73,74 Whitman et al73 showed that administration of exogenous IL-18 increased atherosclerotic lesion size and increased the number of lesion-associated T cells in apolipoprotein (apo) E–deficient mice without altering serum cholesterol concentrations. If the action of IL-18 was inhibited, using a murine IL-18 binding protein, fatty streak development was prevented and plaque progression was delayed in apolipoprotein E–deficient mice.74

The proinflammatory IL-18 may participate in all stages of plaque development. With the development of a fatty streak and the transmigration of monocytes into the intima, IL-18 secretion is promoted by TNF-α and IL-1β cytokines generated by the local inflammatory response.68 Enhanced IL-18 secretion in turn promotes IFN-γ production by both macrophages and SMCs, culminating in the further production of TNF-α and IL-1β.67,69 IL-18–induced IFN-γ production promotes the development of a Th1 immune response, with Th1 cells further producing IFN-γ.69 All three cytokines
combined, TNF-α, IL-1β and IFN-γ, enhance IL-18 receptor expression. 67 Hence, a vicious inflammatory circle is created (Figure 3).

Inflammatory cell recruitment into the developing plaque is enhanced by IL-18, which on ligation to its receptor on ECs, induces the expression of the adhesion molecules ICAM-1 and VCAM-1. 67, 75 IL-18 itself can also serve as a chemoattractant for human T cells, possibly promoting their recruitment into the plaque. 76 Furthermore, IL-18 signaling probably promotes processes that may weaken the fibrous cap of a plaque and render it prone to rupture. IL-18 induces the expression of the interstitial collagenases MMP-1 and MMP-13 as well as the gelatinase MMP-9 in human vascular SMCs and macrophages. 67 Thus, IL-18 triggers several proatherogenic functions. As alluded to earlier, blockade of IL-18 action delayed atherosclerotic plaque progression; however, it also led to a stable plaque phenotype by decreasing macrophage, T-cell, and lipid content while increasing SMC and collagen content. 74 Therefore, interventions to limit IL-18 levels may prove beneficial for plaque stabilization and the subsequent reduction of acute thrombotic events. Recently, weight loss has also been shown to reduce circulating IL-18 levels. 77

Conclusions
Over the past few years, it has become increasingly clear that inflammation is at the root of atherosclerosis and its complications. As the mechanisms underlying this process are deciphered, new markers may emerge to assist the clinician in the determination of a patient’s risk for cardiovascular disease. CRP, sCD40L, and IL-18 are three inflammatory markers that result in endothelial activation. Several other novel markers, associated with atherosclerosis, will be highlighted in the second part of this two-part series.

Acknowledgments
This work is supported by the Heart and Stroke Foundation of Canada and the Canadian Institute for Health Research (both grants to Drs Verma and Weisel).

References
56. Schonbeck U, Gerdes N, Vano N, et al. Oxidized low-density lipoprotein augments and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhab-
New Markers of Inflammation and Endothelial Cell Activation: Part I
Paul E. Szmitko, Chao-Hung Wang, Richard D. Weisel, John R. de Almeida, Todd J. Anderson
and Subodh Verma

Circulation. 2003;108:1917-1923
doi: 10.1161/01.CIR.0000089190.95415.9F
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/108/16/1917

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/