Inflammation and Atherosclerosis

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Inflammation plays a pivotal role in all stages of atherogenesis, from foam cell to plaque formation to rupture and ultimately to thrombosis. Insight gained from recent basic and clinical data linking inflammation to atherosclerosis has yielded important diagnostic and prognostic information. Low-grade chronic inflammation as measured by high sensitivity C-reactive protein predicts future risk of acute coronary syndrome independent of traditional cardiovascular risk factors. In addition, individuals with higher "inflammatory burden" gain the largest absolute risk reduction with aggressive risk-lowering therapy. The link between inflammation and atherosclerosis provides a new venue for future pharmacologic agents that may slow the progression of atherosclerosis by inhibiting inflammation.

Introduction

Atherosclerosis, a chronic inflammatory condition characterized by the accumulation of lipids and fibrous elements in subendothelial space, is the leading cause of death in the United States [1•]. As we understand the mechanistic pathways responsible for atherogenesis, the significance of inflammation in this process becomes irrefutable. We now understand that inflammation plays a significant role, from fatty streak formation to plaque rupture to thrombosis. Furthermore, we recognize that an atherosclerotic plaque is not merely a lipid-rich physical blockage, but rather consists of a mix of cytokines, adhesion molecules, chemokines, collagens, elastins, and enzymes secreted from multiple cell types [2].

In light of this, the hypotheses that high-grade stenoses were the sole cause of coronary events have now been challenged by new data from pathologic studies indicating that many events occur in lesions that initially obstruct less than 50% of the coronary lumen [3,4]. Novel noninvasive, invasive, and pathologic techniques have identified unstable plaques that are prone to rupture [5]. There is now a direct link between ongoing chronic inflammation and plaque rupture [6]. In addition, patients with unstable angina have diffuse coronary inflammation regardless of the location of the culprit lesion, indicating that patients with coronary artery disease have elevated degrees of systemic inflammation [7].

Inflammation and Fatty Streak Formation

Atherosclerosis first develops as fatty streaks underlying the endothelium of large arteries, usually appearing within the first decade of life. The process of fatty streak formation begins with diffusion of low-density lipoprotein (LDL) through endothelial cell junctions into the intima, where it undergoes oxidative modification by different pathways [8]. LDL modification can occur by reactive nitrogen or oxygen species generated by enzymes such as myeloperoxidase or 15-lipoxygenase, or through free metal reactions [9,10]. Subsequently, the oxidized LDL can induce endothelial cells to release adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), and increase the expression of chemotactic molecules, such as monocyte chemoattractant protein-1 (MCP-1) [11,12]. These molecules are essential mediators of atherosclerosis.

Under normal homeostatic conditions, endothelial cells are resistant to prolonged contact with blood monocytes. However, in the presence of adhesion molecules such as VCAM-1, P-selectin, and E-selectin, blood monocytes adhere to the endothelial cells and migrate into the subendothelial space. This process, called diapedesis, requires a number of chemoattractants, including MCP-1 [13]. Once located in the arterial intima, monocytes differentiate into intimal macrophages and express a number of scavenger receptors that take up modified lipoprotein particles. The uptake of these oxidized lipoproteins by macrophage scavenger receptors (CD36, SR-A) leads to foam cell formation [14]. Eventually, the foam cells die, contributing to fatty streak formation and inflammation.

Chronic Inflammation and Lesion Progression

The transition from fatty streaks to more complex atherosclerotic plaques involves smooth muscle cells (SMCs) [15]. Once the fatty streak has developed, SMCs migrate from the medial layer to arterial intima in response to a number of growth factors and cytokines released from macrophages. There, SMCs synthesize extracellular matrix proteins such as collagen and elastin, which contribute to fibrous cap formation [16]. Lesion progression is further influenced by the interactions between monocytes/mac-

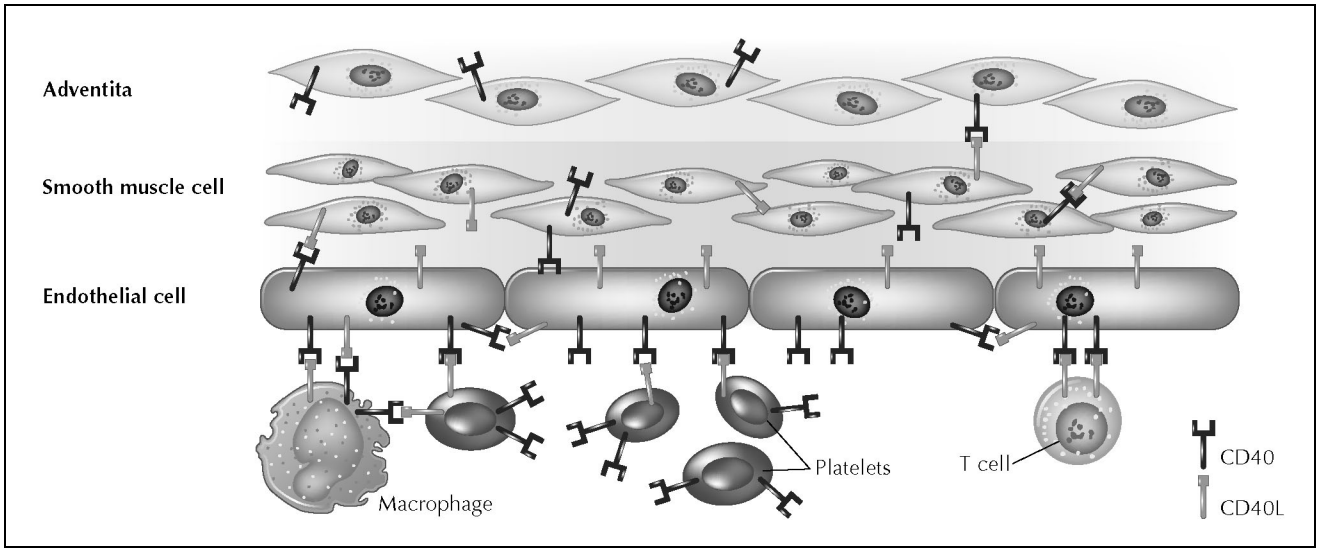

Figure 1. Interaction between CD40 and CD40 ligand is one pathway by which platelets, endothelial cells, macrophages, and other inflammatory cells interact with each other. (*Adapted from* Bhatt and Topol [20•]; with permission.)

rophages and lymphocytes. Lymphocytes, like monocytes, enter the subendothelial space by binding to adhesion molecules, such as VCAM-1, and in response to chemoattractants, such as interferon γ (IFNγ), inducible protein-10, and IFN-inducible T-cell α-chemoattractant (I-TAC) [15]. Once in the arterial intima, the T cell encounters numerous antigens such as oxidized LDL, bacterial and viral antigens, and heat shock proteins. This interaction leads to further elaboration of cytokines such as IFNγ, which has been shown to play a significant role in plaque rupture [17,18].

The interaction of CD40 and CD154 leads to production of mediators such as tissue factor and matrix metalloproteinases (MMPs) [19]. CD40 is not only present on T and B lymphocytes but it is also found on endothelial and smooth muscle cells, as well as on platelets (Fig. 1) [20•]. For example, CD40L-null mice have been shown to have smaller atherosclerotic lesions that are less inflammatory [21]. These observations indicate that both inflammatory and immune reactions are ongoing within the atherosclerotic plaque.

Inflammation and Plaque Rupture

The conventional hypothesis that only high-grade stenoses lead to acute coronary syndrome has changed as more evidence supports the observation that many culprit lesions are initially less than 50%. Data from intravascular ultrasound studies and pathologic specimens revealed that a significant percentage of acute coronary events occur in lesions that are initially not severe on angiographic studies [22].

Three types of physical disruptions lead to plaque rupture and thrombosis [15,23,24]. First, superficial erosion of endothelial cells, which is the result of endothelial cell death or damage from local inflammatory mediators, can lead to plaque rupture. Inflammatory mediators and oxidized lipoproteins can activate the release of enzymes such as MMPs, which degrade the subendothelial basement membrane, leading to subsequent plaque rupture or fissure [25]. Hence, inflammation can promote endothelial loss, leading to exposure of subendothelial collagen and von Willebrand factor to platelets, which results in activation of the coagulation cascade and clot formation. Second, disruption of microvessels within the atherosclerotic plaque may lead to hemorrhage and thrombosis in situ within plaques, eventually causing plaque rupture [26]. Frequent microvessel hemorrhage and thrombosis in the atherosclerotic plaque stimulates SMCs, leading to migration and proliferation of SMCs into the intima. These frequent events can lead to plaque progression and eventual plaque rupture [27]. The mechanism of microvessel formation within the atherosclerotic plaques involves inflammatory cells within the plaque in addition to growth factors secreted from SMCs. For example, macrophages within the atherosclerotic plaque produce angiogenic mediators, such as basic fibroblast growth factor and vascular endothelial growth factor (VEGF) [28]. These mediators can stimulate angiogenesis and microvessel formation. Furthermore, administration of angiogenesis inhibitors prevents microvessel formation and plaque evolution in mice [29], providing even greater support for these hypotheses. The third mechanism of plaque disruption is rupture of the plaque's fibrous cap and subsequent exposure of tissue factor to coagulation factors. As mentioned previously, Th1 cells secrete many proinflammatory cytokines, including IFNγ, which inhibits collagen synthesis. Furthermore, activated macrophages release proteases such as collagenase, gelatinases, and MMPs, which can break down collagen and elastin [30,31]. In addition, many other inflammatory elements, such as macrophage colony-stimulating factor

(M-CSF), MCP-1, and interleukin-1 (IL-1), further fuel this process by attracting and stimulating the macrophages to release proteases [32]. Eventually, the breakdown of collagen and elastin can weaken the fibrous cap, rendering it more susceptible to rupture.

Inflammation and Thrombosis

Under normal homeostatic conditions, the fibrinolytic mechanisms outweigh the procoagulant pathways, hence preventing the progression of plaque rupture to a clinically significant coronary event. In fact, 9% of healthy individuals and up to 22% of diabetic and hypertensive subjects have asymptomatic disrupted plaques in their coronary arteries [33]. Platelet activation is central to formation of thrombus [20•]. Under normal conditions, the intact endothelium prevents platelet activation; however, when the atherosclerotic plaque is ruptured or fissured, circulating platelets are exposed to subendothelial collagen and von Willebrand factor. Furthermore, plaque rupture leads to exposure of tissue factor to coagulation factor VII, leading to activation of both intrinsic and extrinsic blood coagulation cascades. These interactions lead to platelet activation and thrombosis [34]. Interestingly, inflammation plays a significant role in all these pathways.

Under certain inflammatory conditions, mononuclear phagocytes upregulate the tissue factor gene, leading to tissue factor expression in the atheroma. Recent data indicate that the interaction of CD40 and CD40 ligand plays a role in thrombosis (Fig. 1) [20•,35]. These interactions not only stimulate adhesion molecules, chemokines, and MMPs, but they also induce the expression of tissue factor [36,37]. Furthermore, platelets can produce a number of inflammatory mediators, which further propagates the cycle between inflammation and thrombosis. For example, platelets produce platelet-derived growth factor, platelet factor 4, CD40 ligand, thrombospondin, transforming growth factor-β, and nitric oxide, all of which have been shown to be involved in atherogenesis and thrombosis [38,39]. In addition, the close interaction between neutrophils and platelets and monocytes and platelets leads to leukocyte activation, cell-adhesion molecule expression, and generation of signals that promote integrin activation and chemokine synthesis [40,41]. All of these reasons provide further support for the close interaction between thrombotic and inflammatory pathways. Additionally, blood neutrophil-platelet and monocyte-platelet aggregates correlate with coronary disease activity [42,43].

Inflammatory Genes and Atherosclerosis

Numerous animal studies have provided evidence for the close link between inflammation and atherosclerosis [44]. For example, knockout animal models that lack M-CSF, MCP-1, or IFNγ have shown a significant reduction in lesion area [45].

Risk Factors for Inflammation in Atherogenesis

A number of factors play a role in triggering inflammation. For example, oxidized lipoproteins, dyslipidemia, hypertension, diabetes, obesity, and infections all may play a role [30].

The oxidative modification of LDL and its significance in initiating atherosclerosis has been studied extensively [8,46]. The modification of native LDL to its oxidized form has a number of inflammatory and immunologic implications [45]. Oxidized LDL can increase monocyte adhesion, monocyte and T-cell chemotaxis, scavenger receptor A expression, CD36 expression, foam cell formation, and induce proinflammatory genes through nuclear factor κB (NF-κB), AP-1, and *c*-amp. Furthermore, oxidized LDL can induce cellular and humoral immune responses, increase apoptosis and necrosis, and lastly enhance procoagulant activity by inducing tissue factor expression and increasing platelet aggregation [45]. Interestingly, many of the pathways that are involved in lipid modification are now known [47] and have been shown to be associated with coronary artery disease presence [48•].

Among other lipoprotein particles, high-density lipoprotein (HDL) cholesterol has gained significant attention not only for its reverse cholesterol transport properties, but also for its antioxidant activity. HDL particles can transport antioxidant enzymes such as paraoxonase [49], which can neutralize the proinflammatory effects of oxidized lipids. Other lipoprotein particles, such as very low-density lipoprotein (VLDL), may have proinflammatory and atherogenic properties [50].

Diabetes is another significant risk factor for atherosclerosis, which acts through inflammatory and noninflammatory pathways. Through nonenzymatic glycation of macromolecules, advance glycation end-products (AGE) bind with their receptor. These AGE-associated modifications can lead to increased expression and production of inflammatory mediators and proinflammatory cytokines [51,52]. In addition, the diabetic state promotes oxidative stress mediated by reactive nitrogen and oxygen species [53]. These oxidative pathways lead to modification and functional modulation of other macromolecules. Furthermore, many of these oxidative pathways can consume nitric oxide, a potent vasodilator secreted by endothelial cells, thereby leading to endothelial dysfunction.

Inflammation also provides a mechanism by which hypertension is linked to atherosclerosis. Angiotensin II, a potent vasoconstrictor, stimulates production of superoxide anion, which is a common pathologic consumer of nitric oxide in the vessel wall [54]. In addition, angiotensin II can also increase the expression of proinflammatory cytokines such as IL-6, MCP-1, and VCAM-1 [55–57]. Therefore, hypertension provides a link between inflammation and atherosclerosis.

Lastly, *Chlamydia pneumoniae* and *Helicobacter pylori* have been found in atherosclerotic plaques [58]. Intravascular infection can lead to local inflammation, which can further promote atherosclerosis, suggesting that antibiotics

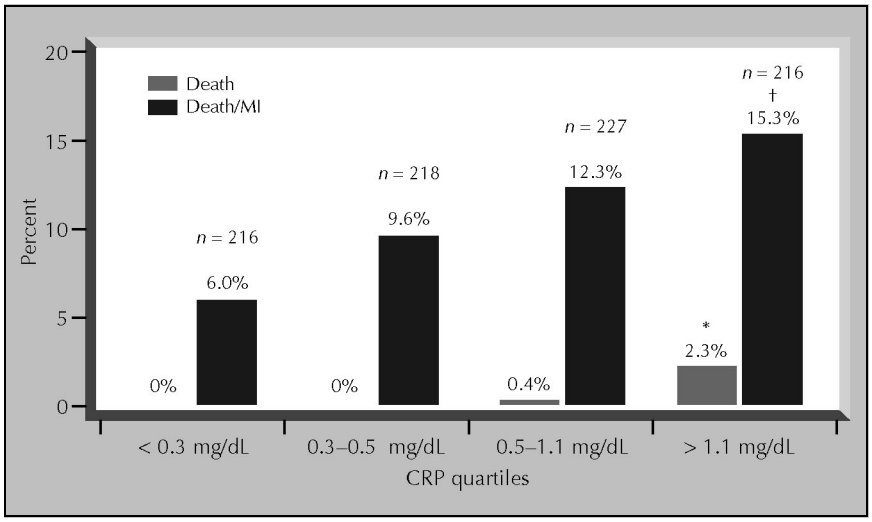

Figure 2. Data show a dramatic step-wise increment in death or death and myocardial infarction (MI) with each increasing quartile of C-reactive protein (CRP). Individuals in the fourth quartile of CRP had an absolute 9.3% increase in the combined endpoints of death and MI compared with those in the first quartile. (*Adapted from* Chew *et al*. [71•]; with permission.)

may have a beneficial role in preventing atherosclerosis. However, clinical trials looking at the effect of antimicrobial agents on clinical outcome have failed to show a consistent benefit [59–61].

Inflammatory Markers and Atherosclerotic Risk

Our new understanding of the pivotal role of inflammation in atherogenesis has raised interest in finding easily measured markers, which can assess the "inflammatory burden." Furthermore, given the fact that a significant number of myocardial events occur in individuals with "normal" LDL cholesterol, a marker that could identify these individuals at risk of coronary events would be of significant interest [62•].

Among the inflammatory markers, such as serum amyloid A (SAA), leukocyte count, fibrinogen, high sensitivity C-reactive protein (hs-CRP), nitrotyrosine, and myeloperoxidase, that have been investigated, hs-CRP has the most stability, assay precision, accuracy, and availability to date [48•,63,64].

C-reactive protein is not only a marker of inflammation, but is also a mediator. Numerous studies show evidence that CRP plays a direct pathogenic role in atherogenesis. CRP can activate complement, enhance T-cell–mediated endothelial cell destruction [65,66], induce expression of adhesion molecules such as VCAM-1 and E-selectin [65], stimulate macrophages to produce tissue factor [67], attenuate nitric oxide production [68], increase plasminogen activator inhibitor-1 expression and activity in human endothelial cells [69], inhibit angiogenesis, and promote intima-medial thickening in children [70]. All of these provide a direct link between CRP and atherothrombosis.

Numerous epidemiologic studies in individuals without coronary artery disease (CAD) demonstrate that hs-CRP levels predict future cardiovascular events [62•]. In addition, the association between CRP levels and future cardiovascular events has been independent of age, smoking, cholesterol levels, diabetes, angiographic CAD, and other major cardiac risk factors. Furthermore, CRP levels predict recurrent ischemia and death in individuals with stable and unstable angina, those undergoing coronary intervention [71•], and patients presenting with an acute myocardial infarction (Fig. 2) [62•,72–75].

C-reactive protein has additive predictive value when combined with LDL cholesterol level or Framingham Risk Score [62•,75]. In addition, CRP levels identify individuals at elevated global risk in whom LDL cholesterol levels are not elevated. Ridker *et al*. [75] showed that CRP levels were a stronger predictor of risk than LDL cholesterol in over 27,000 healthy American women followed over a mean of 8 years. Lastly, CRP adds prognostic information to features of metabolic syndrome and can predict the development of type 2 diabetes mellitus and symptomatic peripheral arterial disease [69,76•,77].

On March 14 and 15, 2002, a workshop entitled "CDC/AHA Workshop on Inflammatory Markers and Cardiovascular Disease: Application to Clinical and Public Health Practice" convened in Atlanta, GA to address the growing number of publications relating inflammatory markers to cardiovascular disease [76•]. The goals of this workshop were to identify the best available test, define patients who should be tested, identify conditions in which the test would be useful, and implement the criteria used to define high-risk patients.

Based on the Centers for Disease Control/American Heart Association (CDC/AHA) guidelines, hs-CRP is the inflammatory marker of choice for cardiovascular risk stratification. However, it should not be performed in individuals with underlying inflammatory or infectious conditions for the purpose of risk stratification. An hs-CRP level of less than 1.0 mg/L is considered low risk, 1.0 to 3.0 mg/L intermediate risk, and greater than 3.0 mg/L high risk. These cut-points are based on the distribution of hs-CRP in over 40,000 persons from over 15 populations.

The Writing Group endorsed the optional use of hs-CRP in patients at intermediate risk (10% to 20% risk of

coronary heart disease [CHD] over 10 years). Physicians who may need more information in order to guide their decision in regard to further diagnostic testing or therapy may use hs-CRP level as an additional tool. According to the Writing Group, individuals with 10-year risk of greater than 20% would not benefit from hs-CRP measurements because they already qualify as CHD risk equivalent and require aggressive medical therapy and lifestyle management. At this time, treatment of elevated hs-CRP solely on the basis of the hs-CRP levels is not recommended.

The hs-CRP assay also may be used for prognostic purposes in secondary prevention. However, the Writing Group sees the utility of hs-CRP in secondary prevention as somewhat limited at this time because these patients should be treated aggressively regardless of their CRP levels. Furthermore, the Writing Group discouraged the use of serial testing for hs-CRP as a means to monitor therapy or to measure disease activity.

The recent guidelines issued by this group are a dramatic advancement in the new paradigm of global risk assessment. However, new advances in medicine come slowly; we, therefore advocate testing of hs-CRP in all individuals at intermediate or high risk for cardiovascular disease [78•].

We believe hs-CRP should be measured in conjunction with a cholesterol panel, and the results should be used to help clinicians in risk stratification in both primary and secondary prevention [48•,79]. Multiple deleterious lifestyles and behaviors contribute to the majority of deaths from cardiovascular causes. Compliance with lifestyle recommendations is directly related to the absolute risk perceived by the individuals. Thus, the addition of hs-CRP to traditional risk factors will provide an improved prediction tool, which should be shared with patients for better compliance with lifestyle and behavioral changes.

Therapeutic Interventions to Treat Inflammation in Atherosclerosis

Numerous therapeutic agents have shown significant reduction in morbidity and mortality associated with CAD. Among these, 3-hydroxy-3-methylglutaryl coenzyme A inhibitors (*ie*, statins), aspirin, angiotensin-converting enzyme (ACE) inhibitors, and peroxisome proliferator activated receptor-γ (PPARγ) agonists have shown anti-inflammatory affects [80,81•,82•,83].

Multiple primary and secondary prevention trials of statins have demonstrated a decrease in morbidity and mortality from cardiovascular causes. Although statins decrease LDL cholesterol, it is becoming increasingly clear that the beneficial effects of statins cannot be fully explained by the lipid-lowering effects of these drugs. Recent studies indicate that statins reduce the number of inflammatory cells within an atherosclerotic plaque, decrease superoxide production from NADPH oxidase in vascular SMCs, and upregulate endothelial nitric oxide synthase (eNOS), leading to increased nitric oxide (NO) formation [84–87]. These and other actions of statins collectively promote an anti-inflammatory response and suggest that statins may function as potent anti-inflammatory agents [88•]. Furthermore, in two recent randomized trials, the Cholesterol And Recurrent Events (CARE) [89] and the Air Force/Texas Coronary Artery Prevention Study (AFCAPS/TexCAPS) [90], the benefit associated with statin use in patients with elevated CRP was much greater than those with low CRP levels, indicating that individuals with elevated CRP levels may benefit more from statin therapy.

Aspirin exerts both antiplatelet and anti-inflammatory properties. It has been shown to have a significant impact on decreasing morbidity and mortality associated with CAD. Furthermore, in patients with CAD it has been shown to reduce CRP (in some studies) and other inflammatory markers. Similar to statins, patients with elevated CRP levels gain the most risk reduction with aspirin treatment [91].

Angiotensin-converting enzyme inhibitors are another class of drugs with significant reduction in cardiovascular morbidity and mortality. However, the reduction seen in mortality with these agents goes beyond blood pressure lowering. As alluded to previously, angiotensin II, a potent vasoconstrictor, also stimulates proinflammatory cytokines. For example, it can stimulate IL-6, MCP-1, and increase the production of reactive oxygen species from vascular cells [92–94]. It is, therefore, no surprise that ACE inhibitors exert anti-inflammatory effects. In fact, the ACE inhibitor quinapril has been shown to decrease the expression of MCP-1, IL-8, and NF-κB in a rabbit model of atherosclerosis [95].

Thiazolidinediones (TZDs) and PPARγ agonists are insulin sensitizers used to treat diabetes mellitus. PPARγ agonism has been shown to have anti-inflammatory activities, including inhibition of VCAM-1 and intercellular adhesion molecule-1 (ICAM-1) expression from activated endothelial cells [96]. In addition, TZDs have been shown to reduce CRP and MMP-9 [83]. However, the effect of TZDs on inflammation and cardiovascular endpoints in patients is still under investigation.

Clopidogrel, an adenosine diphosphate (ADP) receptor blocker, also exerts antithrombotic and anti-inflammatory properties. For example, CD40 ligand plays a significant role as a mediator between platelets and other cells such as endothelial cells and monocytes. This interaction leads to release of chemokines and adhesion molecules, which can further promote leukocyte recruitment to sites of vascular injury [35,97]. Clopidogrel may interfere with the platelet-monocyte-endothelial interaction by decreasing the expression of P-selectin and ADP-induced expression of CD40 ligand [20•,98,99]. Interestingly, levels of CD40 ligand have been shown to predict future coronary events in healthy individuals [100]. Furthermore, pretreatment with clopidogrel prior to percutaneous coronary intervention appears to have its greatest benefit in patients with elevated hs-CRP [101•,102••].

Conclusions

Despite the preponderance of basic and clinical data linking inflammation to atherogenesis and the strong association between inflammatory markers such as CRP and cardiovascular risk, randomized trials that prospectively test the arterial inflammation hypothesis are lacking. The most important question is whether lowering the "inflammatory burden" will translate into decreased clinical events.

Recently, we proposed a randomized study of usual care versus CRP-guided therapy in patients with a history of cardiovascular events and an elevated baseline CRP level [81•]. We proposed to serially test aspirin, statins, ACE inhibitors, clopidogrel, fibrates, and TZDs in a step-wise fashion. We believe that this approach of utilizing serial CRP measurement to guide therapy will allow formulation of a rational therapeutic strategy instead of an approach of reflex "polypharmacy" for each individual. This approach will help to answer whether combinations of drug therapy do in fact lead to an incremental decrease in morbidity and mortality.

More recently, two large-scale trials have been implemented to assess the clinical benefit of CRP reduction. The first is a large-scale prevention trial of 15,000 patients without high LDL levels but with elevated CRP levels that was begun in early 2003. This trial, named Justification for the Use of Statins in Primary prevention, an Intervention Trial Evaluating Rosuvastatin (JUPITER), will randomize patients to rosuvastatin or placebo. This study will help to answer whether suppression of inflammatory burden will translate into decreased clinical events [103].

The second trial is the ongoing Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial, which is randomizing patients who fall into the category of high-risk primary prevention or secondary prevention to either clopidogrel or placebo, in addition to aspirin. hs-CRP levels are being measured to ascertain the effect of clopidogrel on CRP and how this correlates with clinical event reduction.

Atherosclerosis and its complications are the leading cause of death in the United States and throughout the world. Our ongoing understanding of inflammation and its contribution to plaque formation and thrombosis have provided a new venue from which we can offer help to our patients. By applying this, we can now 1) use inflammatory markers such as hs-CRP to assess risk, independent of traditional cardiac risk factors; 2) identify individuals who can benefit more from certain medications; 3) design new therapeutic agents that can target novel inflammatory pathways that contribute to atherothrombosis; and 4) identify new pathways by which known drugs may slow the progression of atherosclerosis.

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