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Inflammation and thrombosis in cardiovascular disease

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Abstract

Purpose of the review—This article will summarize recent observations that provide mechanistic insight into the molecular and cellular links between inflammation and thrombosis in the context of cardiovascular and other thromboinflammatory disease states.

Recent findings—Several disease conditions are characterized by a thromboinflammatory state in which interactions of blood cells and components with the vascular wall perpetuate both thrombotic and inflammatory pathways. Targeting these pathways may be of benefit in inflammatory conditions and cardiovascular disease, respectively.

Summary—Ongoing clinical trials should provide additional insight into the hypothesis that the thromboinflammatory state contributes to adverse clinical outcomes.

Keywords

Inflammation; Thrombosis; Cardiovascular disease

Introduction

Atherosclerosis involves a complex interplay between metabolic pathways governing lipid deposition, inflammatory and immune responses to oxidized lipids, and endothelial dysfunction. Myocardial infarction (MI) and stroke ensue when these processes culminate in thrombosis. Markers of inflammation, such as C-reactive protein (CRP), myeloperoxidase (MPO) and leukocyte levels are strong predictors of cardiovascular death, MI, and stroke. Similarly, individuals with chronic inflammatory conditions such as rheumatoid arthritis or systemic lupus erythematous (SLE) are at higher risk for the development and complications of atherosclerosis. This review will summarize recent observations that provide mechanistic insight into the molecular and cellular links between inflammation and thrombosis in the context of cardiovascular disease (CVD), which support the concept of a thromboinflammatory state.

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Platelet-mediated interactions

Platelet activation results in the expression of cell surface receptors, adhesion, and small molecules that can alter the properties of leukocytes, endothelial cells, and other vascular cells [1]. Thus, both by direct contact and through the release of bioactive mediators, platelets may influence the function of surrounding cells. Activated platelets produce eicosanoids and platelet activating factor that have potent effects on inflammation and immunity. Recent work has focused on biologically active cargo released from intracellular platelet granules upon activation. Platelet dense granules store the purine nucleotides ADP and ATP, which have roles in thrombosis, inflammation, ischemia-reperfusion injury, and potentially acute lung injury [2]. Platelet release of serotonin from dense granules may modulate mitogenic and inflammatory responses and has been implicated in neutrophil rolling and adhesion to inflamed endothelium [3]. Finally, inorganic polyphosphates are major components of dense granules that are released during platelet activation; these influence coagulation and inflammation differentially based on the size of the polyphosphate polymer [4, 5]

Platelet α–granules contain adhesive proteins, coagulation, mitogenic and angiogenic factors, CXC and CC chemokines, and other factors. One of the most abundant α-granule proteins, platelet factor 4 (PF4/CXCL4) may influence both neutrophils and macrophages and has been implicated in atherosclerosis and vascular smooth muscle cell (VSMC) responses after injury. PF4 acts at least in part through Kruppel-like factor 4 to transcriptional regulate VSMC, reduce their differentiation and promote an inflammatory phenotype [6].

After translocation from α-granules to the cell surface, P-selectin binds to leukocyte PSGL-1 to initiate physical interactions between the cells. Platelet – leukocyte interactions are then stabilized by β2-integrin-dependent events to promote efficient leukocyte recruitment at the site of vascular injury and the formation of mixed platelet-leukocyte conjugates in circulating blood (Figure 1). Platelet – leukocyte aggregates form and may predict outcomes in acute coronary syndromes (ACS) and following percutaneous coronary intervention (PCI) [7, 8]. In the setting of ACS and PCI, platelet – leukocyte aggregates correlate with the inflammatory marker C-reactive protein (CRP) and with biomarkers of myocardial necrosis, such as troponin [9, 10]. Anti-platelet therapy, in particular with P2Y12 antagonists, has been associated with reductions in platelet-leukocyte aggregate formation. In several clinical studies, use of the P2Y12 antagonist clopidogrel was linked to reductions in P-selectin expression, whereas aspirin was not. The active metabolites of both clopidogrel and prasugrel reduce platelet – leukocyte aggregate formation *in vitro* and in animal models [11, 12]. Anti-platelet therapy has been variably associated with reductions in biomarkers of systemic inflammation, including decreased CRP, CD40L, and P-selectin in a variety of disease states, including: cardiovascular disease, cerebrovascular disease, diabetes, and renal transplantation [13–16]. Reduced expression of IL-1α, 2, 6, 13, 10, TNFα, TNFβ, and CRP has been observed in patients after long-term treatment with clopidogrel. [17] How much of the clinical benefit of anti-platelet therapy derives from reductions in platelet-mediated inflammation is not known.

Strategies to target directly interactions between platelets, endothelial cells, and leukocytes are the focus of ongoing studies. Based on strong data from preclinical animal models, P-selectin appears to be an attractive target to block thromboinflammatory cell interactions. The recombinant monoclonal antibody to P-selectin, inclacumab, was tested in the SELECT-ACS trial in which patients (n = 544) presenting with ACS were randomized to receive placebo, or one of 2 doses of inclacumab (5 or 20 mg/kg) administered as a single infusion [18]. In patients who underwent PCI (n=322), those receiving the higher dose of inclacumab had a reduction in cardiac injury as assessed by plasma levels of troponin I and creatinine kinase MB (CK-MB) levels, although both endpoints barely missed statistical significance (P=0.05). There was also a corresponding decrease in soluble P-selectin levels. The study was underpowered to detect clinical events, however, there were numerically more deaths, MIs, and strokes in the 2 groups that received inclacumab than in the placebo control. Nonetheless, the effects on cardiac biomarkers, and especially the reduction in CK-MB >3 times the upper limit of normal, may portend a clinical value to P-selectin inhibition.

Neutrophil-mediated interactions

Leukocytosis, and in particular elevations in neutrophil count, is a robust predictor of increased morbidity and mortality of ischemic vascular disease. Leukocytosis has emerged as a risk factor for both short term and long term complications in studies of >350,000 patients. Ischemic events themselves elicit an inflammatory response and elevations in white blood count may reflect the extent of injury. However, in the CAPRIE trial of patients with recent MI, stroke or peripheral vascular disease, leukocyte counts obtained within one week prior to a recurrent ischemic event were significantly higher than those observed at baseline or in days 8–120 before an event. Additionally, the degree of accumulation of neutrophils in aspirated thrombus of patients presenting with STEMI is an independent predictor of a poor myocardial blush grade, ST-segment resolution, and left ventricular function at 6 months. [19] These findings suggest that leukocytosis may causally impact the development of ischemic events.

Several regulators of neutrophilia and monocytosis have been proposed to impact the development of CVD. Hematopoietic stem cell proliferation and mobilization result in more monocytes and neutrophils originating from the bone marrow. Additionally, stem cell mobilization to spleen can initiate extramedullary hematopoesis. High density lipoprotein and cholesterol efflux pathways can reverse stem cell mobilization in experimental models [20–22]; whereas hyperglycemia and obesity can promote monocytosis by endogenous danger signals produced by neutrophils[23]. In mice, genetic or pharmacologic approaches that alter coagulation appear to impact atherosclerosis in a neutrophil-dependent manner, and hypercoagulable states are associated with neutrophilia and neutrophil hyper-reactivity [24].

There are numerous ways in which leukocytes impact CVD. On their own, and synergistically with platelets, they generate tissue factor, microparticles, and surfaces for thrombin generation to enhance fibrin formation. Leukocyte homotypic and platelet-leukocyte heterotypic aggregates may cause rheological compromise of microvasculature. Neutrophils and monocytes also promote CVD through the generation of inflammatory

mediators, tissue infiltration, and oxidation of lipoproteins and other proteins. Neutrophil release of the serine proteases elastase and cathepsin G promote efficient thrombus formation in mice by stabilizing fibrin deposition, likely by proteolytic inactivation of endogenous anticoagulants. [25]

In the last several years, evidence has emerged for a key role for neutrophil release of extracellular traps (NETs) in thrombotic conditions. In response to strong stimulation, neutrophils release extrude NETs that consist of DNA and histones in a process that involves histone citrullinated by peptidylarginine deiminase 4, chromatin unwinding, breakdown of nuclear membranes and cytolysis [26]. A key function of the extracellular chromatin material is to entrap and confine microbes to promote their destruction. The chromatin fibers may also serve as a scaffold for clot formation, promote extrinsic and intrinsic activation of the coagulation cascade, and provide resistance to thrombolysis. Several reports indicate a key role for neutrophils and NETs in venous thrombosis in animal models [27–29]. Platelets can trigger NET formation and may also bind to histones to form platelet – NET attachments. Histones activate platelets through TLR-dependent mechanisms to generate the release of polyphosphates [30], which in turn amplify coagulation. NETs have been implicated in experimental transfusion related acute lung injury, where their formation appears to be triggered by activated platelets [31]. Analysis of material obtained by aspiration of coronary thrombi indicated the presence of NETs, histone H1, myeloperoxidase, and neutrophil elastase in samples obtained from fresh clot or following thrombolysis, but not in specimens of organized material. [32] NET formation may also occur in SLE and contribute to premature CVD that occurs in these individuals [33]. Taken together, these findings demonstrate that NETs may serve as a tight link between immunity and thrombosis and suggest novel therapeutic targets for thrombosis. Indeed, in animal models, DNAse1 treatment and peptidylarginine deiminase 4-deficiency dramatically protects against venous thrombosis [34].

Anti-inflammatory therapy in CVD

A number of anti-inflammatory therapies have been tested in patients with acute MI (AMI), however, as-of-yet no therapy has emerged as having a demonstrable benefit in this setting. An example is the development of the complement (C5) inhibitor pexelizumab, which reduces neutrophil count and showed promise in several preclinical studies and a phase 2 trial of individuals with AMI undergoing PCI [35], although this was not the case in a trial of AMI patients receiving thrombolysis [36]. Also, in the APEX-AMI trial, a phase 3 study of 6,000 patients, pexelizumab had no effect on 90-day mortality [37].

The JUPITER trial demonstrated that statin therapy prevented cardiovascular events in individuals with a high inflammatory burden (assessed by CRP levels >2 mg/L), normal LDL-cholesterol values (LDL < 130 mg/dL) and no known CVD. In this patient population, rosuvastatin at a dose of 20 mg daily reduced by 54% the risk of first-ever MI, stroke by 43% [38], and deep vein thrombosis or pulmonary embolism by 43% [39]. Due to the effects of statins on cholesterol metabolism, the JUPITER trial could not definitively establish an anti-inflammatory benefit. However, the lowest clinical event rates were observed in individuals who achieved LDL-cholesterol levels <70 mg/dL and hsCRP levels <2 mg/L.

The effect of anti-inflammatory therapy on vascular events is currently being testing in the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) and the Cardiovascular Inflammation Reduction Trial (CIRT). In CANTOS, individuals with stable coronary artery disease and elevated hsCRP will be randomized to receive the interleukin-1β (IL-1β) inhibitor canakinumab or placebo [40]. CIRT examines the effect of low-dose methotrexate in subjects with type 2 diabetes or metabolic syndrome that have had a heart attack [41]. Both trials will measure rates of recurrent myocardial infarction, stroke, and cardiovascular death. In patients with rheumatoid arthritis, evidence suggests that methotrexate treatment [42] and TNFα inhibitors [43] may improve cardiovascular outcomes. Etanercept, a recombinant fusion protein containing the human TNF receptor that scavenges TNFα, has been investigated in congestive heart failure [44] and administered to a small number of patients with acute MI [45], though with disappointing results.

Anti-thrombotic strategies in inflammation and sepsis

Based on compelling experimental evidence that thrombosis contributes to inflammatory states, several anti-thrombotic strategies have been explored in systemic inflammatory responses and sepsis. After initial promising results in sepsis based on the results of the PROWESS trial [46], activated protein C (drotrecogin α) received FDA approval that was subsequently limited to patients at high risk of death. In the large, international PROWESS-Shock study of critically ill adults with septic shock, drotrecogin α did not reduce mortality at either 28 or 90 days, as compared with placebo [47] and has subsequently been removed from the market. High-dose antithrombin has not demonstrated benefit and is associated with increased bleeding in severe sepsis [48], although it may have efficacy in disseminated intravascular coagulation associated with sepsis [49]. Likewise recombinant TFPI (rTFPI, Tifacogin) failed to improve outcomes in sepsis [50]. Recombinant thrombomodulin has shown promising results in Phase 2 sepsis studies, especially among individuals with coagulopathy and respiratory or cardiac dysfunction, and a phase 3 trial is currently underway [51].

Thrombocytopenia in the setting of sepsis portends poor outcomes for reasons that are not known. Platelets express toll-like receptors (TLRs), including TLR2 and TLR4 that recognize the bacterial peptidoglycan and lipopolysaccharide (LPS), respectively [52–54]. Interactions between LPS and platelet TLR4 have been implicated in sepsis-induced thrombocytopenia, pulmonary fibrin deposition, and microvascular thrombosis in animal models[53]. Platelet activation and sequestration in pulmonary tissue is a key feature in inflammatory states and may contribute to acute lung injury and acute respiratory distress syndrome (ARDS) [55]. Antiplatelet therapy with clopidogrel or prasugel reduces LPS-mediated thrombocytopenia, fibrin deposition in the lungs, and inflammatory mediator up-regulation in mice [12, 53, 56], whereas ticagrelor therapy prevents lung damage and neutrophil infiltration in the cecal ligation puncture model of sepsis in mice (content.onlinejacc.org/data/Journals/JAC/926556/00716.pdf).

Retrospective and observational clinical studies hint that anti-platelet therapy may improve outcomes in critical illness, especially in individuals at risk for acute lung injury. In a single center experience of 224 patients hospitalized with pneumonia requiring intensive care,

those who were taking antiplatelet therapy (aspirin, clopidogrel, or ticlopidine) had shorter lengths of stay [56]. A subsequent. larger study (n = 615) by the same investigators documented a reduction in mortality in medical and surgical intensive care patients who were receiving aspirin or clopidgorel [57] In population-based observational studies, prehospital anti-platelet therapy was associated with reductions in indices of acute lung injury and ARDS [58–60], although, a more recent publication by one of the groups failed to confirm the benefit of aspirin [61]. The ongoing Lung Injury Prevention With Aspirin (LIPS-A) trial will test the hypothesis that early aspirin (325 mg followed by 81 mg daily) given within 12 hours of hospitalization to individuals at risk for acute lung injury will improve outcomes [62].

Conclusions

Substantial mechanistic evidence links inflammation and thrombosis. Observations in preclinical animal models indicated the existence of thromboinflammatory conditions and suggest that targeting inflammatory and thrombotic pathways may prove benefical. Ongoing clinical trials should determine the clinical efficacy of anti-inflammatory therapy in reducing the thrombotic complications of cardiovascular disease and better define the role of anti-thrombotic and anti-platelet therapy in inflammatory states. Another promising strategy may be to reduce leukocyte counts, rather than targeting their function. Identification of the ideal agents, their timing and duration of administration await future studies.

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Key Points

- Emerging evidence suggests the existence of thromboinflammatory disease states in which platelets and leukocytes interact and generate novel mediators of inflammation and thrombosis
- A recent trial targeting P-selectin in acute myocardial infarction holds promise for reduction in ischemic damage during coronary interventions
- Anti-platelet therapy has been shown to variably reduce systemic markers of inflammation and is being studied for clinical efficacy in ongoing trials
- Ongoing clinical trials are evaluating the benefit of anti-inflammatory therapy in CVD and strategies aimed at lowering leukocyte count may prove to be a viable method of targeting inflammation and thrombosis

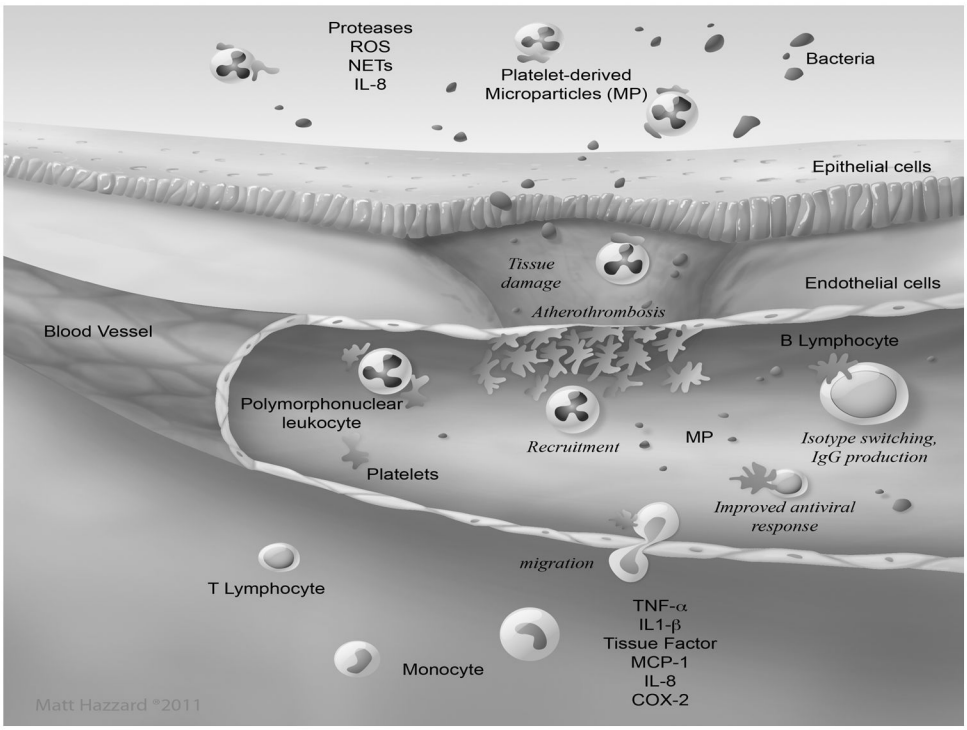

Figure 1. Contribution of platelet–leukocyte interactions to a thromboinflammatory state

Platelets are activated at the site of endothelial damage or in the microcirculation of infected/inflamed tissue. Activated platelets bind leukocytes to form heterotypic complexes, and communicate signals that result in a variety of specific responses. Platelets mediate the recruitment of leukocytes at the site of atherosclerosis or thrombus formation, as well as in inflamed tissue. In some cases, these interactions mediate platelet–leukocyte co-migration across the mucosal epithelium. In this way, platelets contribute to the promotion of inflammatory reactions, which, when not controlled, can exacerbate tissue damage. Platelets might support lymphocyte homing in peripheral lymph nodes, stimulate isotype switching and production of IgG by B lymphocytes, and might help lymphocyte responses to viruses and neutrophil response to bacteria. In this way, platelets contribute to host defense. Illustration by Matt Hazzard, University of Kentucky, Information Technology.