Editorial

Thrombosis and Inflammation: A Question in Need of an Answer

Thrombovascular disease includes arterial and venous occlusion, with advancing age as the single most important risk factor[1]. Under the age of 40 years the most common presentation of vascular occlusion is deep venous thrombosis[2], while in older populations arterial thrombosis predominates. Atherosclerosis is the leading cause of arterial thrombotic events such as myocardial infarction, stroke, or peripheral vascular disease. Arterial occlusion is precipitated by rupture of an unstable atherosclerotic plaque. Plaque rupture establishes a prothrombotic state resulting in thrombus extension into the vessel lumen and plaque[3,4].

A number of congenital and acquired risk factors are associated with venous thrombosis. Familial or acquired protein S, protein C and antithrombin III deficiency, as well as germline mutations such as factor V Leiden and prothrombin G20210A predispose to venous thrombosis. Additional risk factors include lower extremity surgery[5] and malignancy[6].

A positive relationship between thrombosis and inflammation[7] has been reported in various clinical settings. In sepsis the synergistic interaction between inflammation and coagulation is well recognized. The benefit of activated protein C in the treatment of sepsis arises from both its antiinflammatory and anticoagulant properties[8]. In cardiovascular disease elevated inflammatory markers are associated with an increased risk of event. In the antiphospholipid syndrome (APS) recent experimental data have also implicated inflammation in thrombus formation.

In this issue of *The Journal* the study by Sailer and colleagues examines the association between inflammation and thrombosis in APS[9]. Given that lupus anticoagulant (LAC) positivity alone does not guarantee the incidence of thrombosis, the authors speculate that additional risk factors, such as systemic inflammation, may contribute to thrombus formation in this patient population.

In their study, levels of inflammatory markers were measured in 2 LAC-positive populations and a control group. One LAC group had evidence of previous thrombotic events, while the other group remained event-free. Blood collection was done at varying intervals after thrombotic event in those with previous events.

It should be noted that the design of this study is purely descriptive, and no predictive associations can be tested, as the measurement of exposure (inflammatory markers) is done after the outcome of interest (the thrombotic event). The authors found that LAC positivity was associated with an inflammatory state when compared to controls. However, there was no significant difference in the level of inflammatory markers between the 2 LAC groups, including C-reactive protein (CRP), fibrinogen, and factor VIII. The authors imply that inflammatory markers cannot distinguish between LAC patients with and without previous thrombosis and conclude that "inflammation might not have a relevant impact on the development of thrombosis in patients with LAC"[9]. Unfortunately, this statement cannot be made safely in the context of a descriptive study design and should be tested again in a well-powered prospective predictive study. Further, these findings stand in contrast to current evidence suggesting a strong causal link between inflammation and thrombosis[7,10,11] and therefore merit serious consideration. What we learn from this study is that although these markers do not discriminate after the fact between LAC groups with versus those without thrombosis, LAC positivity is associated with abnormal levels of markers of inflammation when compared to controls.

The relationship between inflammation and atherosclerotic cardiovascular disease has been extensively studied. An increased risk of cardiovascular events is associated with elevated plasma levels of inflammatory markers such as C-reactive protein (CRP) and fibrinogen[10,12,13].

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Connective tissue diseases such as rheumatoid arthritis and systemic lupus erythematosus are associated with increased cardiovascular risk, with both recognized as independent risk factors for accelerated atherosclerosis[14,15]. This association is independent of the confounding presence of antiphospholipid antibodies (aPL) that also pose a risk for arterial thrombosis.

Taken together, these studies suggest that chronic inflammation is a potent mediator of atherosclerosis and cardiovascular disease. Recent evidence demonstrates that an acute inflammatory insult, such as infection, transiently increases the risk of myocardial infarct and stroke[16].

The question remains whether specific inflammatory mediators are solely markers of systemic inflammation or are directly involved in the pathogenesis of atherosclerotic disease. Recent studies have demonstrated expression of CRP within the atherosclerotic plaque. Within the plaque, CRP plays a multiplicity of roles, influencing key promoters of atherosclerosis (e.g., endothelium, monocytes, low density lipoproteins, complement, and cytokines). This evidence suggests that the relationship between CRP and cardiovascular events represents more than an epiphenomenon[17].

APS is the quintessential thrombotic disease, typically presenting with either venous or arterial thrombosis. APS animal models yield insight into the pathogenesis of APS and the interaction between coagulation and inflammation in this disease process. In murine models of APS-mediated thrombosis aPL obtained from symptomatic APS patients are transferred into recipient mice. In studies of arterial thrombosis in which mice are subjected to direct endothelial injury, aPL are associated with larger thrombi and a prolonged total clot time (time from clot formation to dissolution). The ability of passively acquired antibodies to mediate disease in a nonsusceptible host strongly argues for the direct pathogenicity of aPL antibodies[18].

aPL antibodies appear to mediate their pathogenic effect through direct complement fixation and activation. Activation of complement C3 is an essential requirement for aPL-mediated thrombus formation, with inhibition of C3 convertase activity restoring post-injury thrombotic response to control levels[18]. Further studies have shown that activation of C5 is required for aPL-induced thrombosis. The presence of an anti-C5 monoclonal antibody negates the prothrombotic effects of aPL antibodies, with thrombus size and total clot time reduced to control levels[19]. The importance of complement activation has been definitively established in animal models of APS-mediated pregnancy loss[20]. In this model system, activation and cleavage of C5 results in marked neutrophilic infiltration of decidual tissue with neutrophil depletion, preventing fetal loss. By extension, neutrophil recruitment likely contributes to the pathogenesis of systemic thrombosis. This body of work suggests that aPL-mediated complement activation results in a robust inflammatory response, culminating in stereotypical clinical outcomes.

As discussed above, despite experimental evidence supporting a link between inflammation and thrombus formation in the APS, Sailer and colleagues fail to identify this relationship in a clinical context[9]. This apparent discrepancy may reflect timing of the measurement of the inflammatory markers in relation to the timing of the thrombotic event, as inflammatory markers may fluctuate over time.

Current models suggest that thrombus formation is an acute event precipitated by antibody binding, complement activation, and recruitment of inflammatory mediators. In this model, thrombus formation may be accompanied by a transient rise in inflammatory markers. By analogy to myocardial infarction, biochemical evidence of this inflammatory burst would likely be short-lived, although clinical evidence of the event would persist. Perhaps obtaining measurements of pertinent markers in a more temporally relevant manner would be more illuminating as to the interaction between inflammation and thrombus formation in the APS.

An alternative interpretation is that APS-associated inflammation may be limited to the site of antibody binding. This would be in keeping with the restriction of thrombus to a specific vascular bed rather than a disseminated thrombotic process. In this scenario, markers of systemic inflammation may not accurately reflect the extent of inflammation at a specific site. Again, determining the level of inflammatory markers at timepoints flanking a thrombotic event could clarify this question.

Sailer and colleagues demonstrate that elevated inflammatory markers associate with autoantibody positivity rather than thrombosis. They infer that chronic inflammation does not appear to play a contributory role in antibody-mediated thrombus formation. The current model of APS-mediated thrombosis supports this conclusion, since antibody binding and complement activation would precede recruitment of inflammatory mediators. Thus, inflammation is not an inciting event, but instead a consequence of antibody binding and activation of the complement cascade. In turn, inflammatory mediators may promote a prothrombotic state through endothelial injury and release of tissue factor.

Given the current pathogenesis model for APS assays of complement, cleavage products may correlate more closely with thrombosis, particularly at the time of the acute event. Indeed, sequential determination of complement cleavage products may be able to predict an imminent thrombotic episode. Additional studies will be required to clarify this possibility.

The treatment of APS reflects a noninflammatory paradigm, with warfarin as the mainstay of treatment. In the February issue of *The Journal*, Erkan, *et al* presented current recommendations regarding warfarin use in APS, as well as possible alternatives, for the prevention of secondary thrombosis[21]. Clearly, the role of specific inhibitors of complement in the treatment of the APS needs to be studied, as

they are potential therapeutic agents in the primary and secondary prevention of thrombosis.

The synergistic relationship between inflammation and thrombosis is complex. The question that remains is the significance of the identified chronic inflammatory state associated with LAC positivity. More studies are needed to determine the significance of inflammation in APS, and whether what is learned will translate into useful therapeutic information. These studies will have to be adequately powered to address the issue of the timing of measurement of exposure and the outcome.

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