Novel Considerations in the Pathogenesis of the Antiphospholipid Syndrome: Involvement of the Tissue Factor Pathway of Blood Coagulation

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ABSTRACT

The antiphospholipid syndrome (APS) is characterized by clinical manifestations such as venous and arterial thrombosis, thrombocytopenia and/or recurrent pregnancy loss, as well as the persistent presence of laboratory markers of antiphospholipid (aPL) antibodies detected in laboratory assays. Though it is generally accepted that aPL antibodies, such as anticardiolipin (aCL), anti–β2 glycoprotein I (anti-β2GPI), and lupus anticoagulants (LA) contribute to the pathogenesis of APS, precise mechanism(s) are yet to be fully described. It is probable that aPL antibodies bind to a range of cellular targets (e.g., platelets, endothelial cells, and monocytes), leading to thrombosis and obstetric complications. There is now increasing evidence that alterations to the tissue factor (TF) pathway of blood coagulation contribute toward hypercoagulability in patients with aPL antibodies. This article reviews current evidence that suggests changes and/or interference to the major pathway of blood coagulation may represent a novel mechanism that contributes to the development of APS.

KEYWORDS: Tissue factor, antiphospholipid syndrome, antiphospholipid antibodies, tissue factor pathway inhibitor, thrombosis

The antiphospholipid syndrome (APS) is a complex, acquired autoimmune disease characterized by clinical manifestations of thrombosis, recurrent pregnancy loss and/or thrombocytopenia, coupled with the persistent laboratory detection of antiphospholipid (aPL) antibodies such as anticardiolipin (aCL), anti–β2 glycoprotein I (anti-β2GPI), and lupus anticoagulants (LA).[1] APS is associated with significant morbidity and mortality, and despite recent advances in classification and laboratory testing, the disease still currently represents a considerable challenge in terms of both diagnosis and treatment. Although it is generally accepted that aPL antibodies contribute to the clinical manifestations of APS, how this occurs remains to be clearly resolved. It is probable that there are a myriad of intravascular and cellular interactions that contribute to the development of APS rather than a single triggering event. Such mechanisms that have been well described include aPL antibody interference to the protein C anticoagulant pathway,[2,3] interaction of aPL antibodies

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with endothelial cells resulting in increased tissue factor (TF) expression,[4,5] and promotion of platelet aggregation after aPL antibody binding.[6] A full review of the major proposed pathogenic mechanisms is available elsewhere in this issue of *Seminars in Thrombosis and Hemostasis*.[7] Whereas these mechanisms contribute to the understanding of APS pathophysiology, new mechanisms implicated in the development of APS continue to be described, including involvement of the TF pathway. The aim of this article is to focus on and review the contribution of the TF pathway toward the pathophysiology of APS.

TISSUE FACTOR PATHWAY

The TF pathway is the major physiologic trigger of blood coagulation. After vessel or tissue injury, the 45-kDa integral membrane protein TF is exposed to the circulation and triggers a sequence of reactions culminating in thrombin generation and fibrin formation that helps to stabilize the platelet plug. Blood coagulation has three major phases: (1) *initiation*, where TF is exposed to the circulation after vessel damage to generate trace amounts of thrombin. (2) *Amplification*, where thrombin is central to overcoming the inhibitory effect of the natural regulator of the TF pathway, tissue factor pathway inhibitor (TFPI). This is achieved by activation of platelets and other coagulation factors (i.e., factors XI, V, and VIII) that are associated with phosphatidylserine on damaged endothelial cells or activated platelets to generate further thrombin. (3) *Propagation*, where thrombin generation leads to the formation of fibrin monomers that help to stabilize the platelet plug and to form a clot[8] (Fig. 1).

TF was once thought to be entirely extravascular, providing a "hemostatic envelope" and protection against vascular injury. Whereas TF is easily expressed in cultured endothelial cells in vitro using a variety of

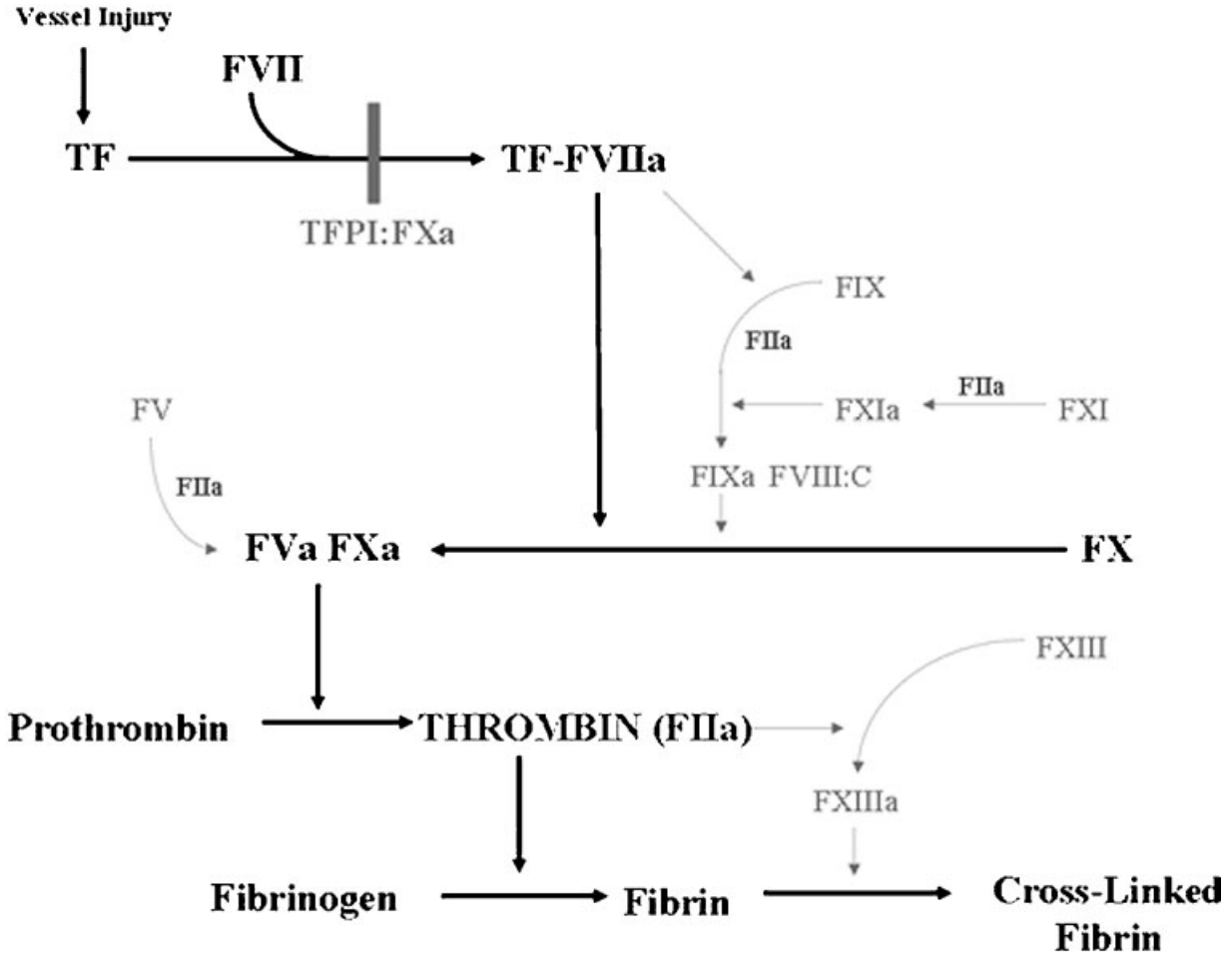

Figure 1 Model of blood coagulation. The tissue factor pathway (indicated in boldface) is triggered after injury to a blood vessel or tissue with the exposure of TF to the circulation resulting in the formation of the TF-FVIIa complex *(initiation phase)*. This complex generates trace amounts of thrombin, which is essential for feedback amplification of other coagulation factors to (i) overcome the inhibitory effect of TFPI and (ii) generate more thrombin *(amplification phase)*. Increased thrombin generation results in fibrin monomer formation and eventually cross-linking of monomers to form the stable fibrin polymer *(propagation phase)*.

agents, it remains controversial whether endothelial cells constitutively express TF in vivo.[9] Recently, there has been intense focus and debate on the physiologic role(s) and measurement of plasma-borne TF microparticles derived from various cells such as monocytes, endothelial cells, platelets, and smooth muscle cells. TF microparticles have been reported to be elevated in a range of diseases, including cardiovascular disease[10,11] and various cancers,[12,13] indicating that they may contribute significantly to thrombosis. It may therefore be reasonable to suggest that TF microparticles have an important role in maintaining thrombin generation during normal hemostasis.

The major natural regulator of the TF pathway is TFPI, a 42-kDa, three-domain, Kunitz-type protease inhibitor constitutively produced by microvascular endothelial cells.[14] TFPI acts in a factor Xa (FXa)-dependent manner to inhibit TF-FVIIa complexes on phospholipid surfaces and thus regulates thrombin generation and fibrin formation. To date, TFPI deficiency has yet to be clearly associated with either alterations in genotype or with thrombotic phenotype. This is probably due to heterozygous expression of TFPI being sufficient to maintain normal hemostasis. There is also doubt over the suitability of current assays for TFPI in that they (i) only measure the plasma pool accounting for ~10 to 50% of total body TFPI and (ii) have demonstrated poor correlation between different methods.[15–17]

THE TF PATHWAY IN APS

There is increasing evidence to suggest that changes to the TF pathway of blood coagulation contribute to the development of thrombosis in APS. A role for the TF pathway in APS was first implicated with experiments that demonstrated that incubation of serum from patients with systemic lupus erythematosus enhanced the procoagulant activity of cultured human endothelial cells,[3,18–20] with aPL antibodies from these patients thought to be responsible for the increase in procoagulant activity. Similarly, there is considerable evidence suggesting that TF activity on monocytes is stimulated by aPL antibodies from patients with APS[4,5,21,22] and that monocytes from APS patients exhibit increased expression of TF and TF mRNA.[4,21] Increased plasma levels of TF antigen in APS patients have been reported, consistent with a hypercoagulable state.[4,23] Furthermore, TF microparticles have been reported to be elevated in patients with aPL antibodies,[24,25] though method standardization for the measurement of TF microparticles remains to be resolved.[26] It is probable that aPL antibody–induced TF procoagulant activity on endothelial and other cells (e.g., monocytes), together with increased levels of circulating TF microparticles derived from endothelial cells as a consequence of cell damage and/or other sources, contribute to increased TF activity and thus hypercoagulability in APS.

Whereas data are consistent reflecting increased TF activity in APS, there is no consensus on levels of TFPI in APS. Thus, plasma TFPI levels are reported to be variable in patients with aPL antibodies compared with those of normal healthy individuals.[4,23,27–30] Increased TFPI levels may be a response to increased TF activity, though this does not effectively explain why APS patients have a higher risk of thrombosis. Lower levels of TFPI may be a consequence of TFPI consumption during clotting or interference in the ability of TFPI to regulate coagulation (e.g., anti-TFPI autoantibodies and/or other cross-reacting aPL antibodies).

During the past few years, there have been reports of anti–TFPI-like activity[27,28,31] as well as the identification of anti-TFPI autoantibodies in APS patients.[32] Whether anti-TFPI activity and anti-TFPI autoantibodies reflect the same entity, and/or involve other cross-reacting aPL antibodies, is yet to be elucidated, though given the heterogeneous nature of APS, it would be reasonable to speculate that the anti-TFPI activity reported in these studies represents one or more types of aPL antibodies that bind to and/or inhibit TFPI.

It was reported that anti-β2GPI isolated from APS patients has anti–TFPI-like activity.[33] These authors demonstrated that anti-β2GPI antibodies isolated from the IgG fraction of APS patients caused increased FXa generation in normal plasma containing TFPI compared with that of plasma lacking TFPI. It was hypothesized that anti-β2GPI antibodies compete for the same phospholipid binding sites as TFPI:FXa, thereby interfering with phospholipid-dependent inhibition of FX activation by TFPI.[33] A similar inhibitory component against TFPI was later identified in the IgG fraction of a different group of APS patients that was associated with increased in vitro TF-induced thrombin generation.[27] Further studies by this laboratory demonstrated that purified anti-β2GPI IgG antibodies, but not purified antiprothrombin IgG antibodies, were associated with increased TF-induced thrombin generation in the presence of TFPI, suggesting that anti-β2GPI may bind to and/or inhibit TFPI.[31] Although no cross-reactivity was demonstrated between purified anti-β2GPI and TFPI, these data provide evidence that aPL antibodies impede the TF pathway, possibly through interference to TFPI that could result in the development of hypercoagulability and thrombosis in APS.

Most recently, increased thrombin activity in pooled normal plasma (PNP) supplemented with purified IgG was demonstrated in a subset of patients with LA using the calibrated automated thrombinogram.[34] Inhibition of both in vitro (rTFPI) and in vivo (heparin-induced TFPI plasma levels) TFPI anticoagulant activity was also reported. This study provides further evidence

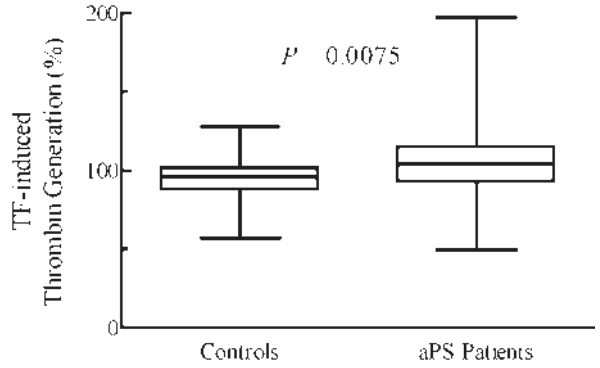

Figure 2 Effect of IgG fractions on TF-induced thrombin generation in pooled normal plasma. IgG from APS patients generates a wider and significantly higher effect than does IgG from controls on TF-induced thrombin generation. Differences between groups assessed using the Mann-Whitney test for non-parametric data. Median (interquartile range): 95.8% (87.5 to 101.7%) for controls and 104.5% (92.8 to 115.8%) for APS patients.

that aPL antibodies such as LA interfere with the normal hemostatic role of the TF pathway and that impairment of the anticoagulant activity of TFPI in particular may be critical in the development of hypercoagulability and thrombosis in APS. It was also demonstrated that IgG from different patients generated variable levels of interference toward TFPI, which could reflect variability in the mechanisms by which aPL antibodies cause thrombosis in different patients. This provides further support that the pathogenesis of APS is likely to be multifactorial, with interference by aPL antibodies to multiple antigenic targets providing an overall net prothrombotic environment.[34] This is consistent with previous data,[27] and indeed, a review of previous results from this laboratory (including those not previously published), again demonstrates a wide range of effects of purified IgG from patients with LA/aPL antibodies on thrombin generation in PNP using a TF-induced thrombin generation assay[35] (Fig. 2). These data support the notion that both procoagulant and anticoagulant mechanisms are affected by aPL antibodies and, significantly, may also reflect the heterogeneous clinical presentations of these patients.

CONCLUSION

Evidence from the past 10 years strongly suggests that abnormalities and/or interference to the TF pathway of blood coagulation may play an important role in the pathophysiology of APS. Of particular significance has been the demonstration that (1) aPL antibodies stimulate TF expression on endothelial cells, (2) TF microparticles are increased in APS patients compared with that of normal controls, (3) anti-TFPI autoantibodies have been identified in APS patients, and (4) anti-TFPI activity is associated with the presence of anti-β2GPI and is correlated with increased TF-induced thrombin generation. As well as continued investigations on the effect of aPL antibodies on endothelial and intravascular cells, future studies will aim to determine the individual and combined contribution of various aPL antibodies toward the TF pathway and the precise relationship between aPL antibodies and TF microparticles. The future may also see the development of target-specific anticoagulant therapy that accounts for specific actions of aPL antibodies on hemostatic parameters, including the TF pathway.

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