Antiphospholipid Syndrome – Pathogenesis

Fariha Saleem
Department of Pathology Holy Family Hospital and Rawalpindi Medical College

Introduction

The antiphospholipid syndrome (APS) is an acquired autoimmune condition characterized by arterial and venous thrombosis, gestational morbidity and presence of elevated and persistently positive serum titers of antiphospholipid antibodies (aPL).\(^1\) In APS, venous thrombosis most commonly manifests as deep venous thrombosis (DVT) or pulmonary embolism (PE), however any part of the venous vasculature may be involved. Cerebral vasculature is the most common site of arterial thrombosis in APS, manifesting as cerebral ischemia or stroke. Myocardial infarction is a less common presentation. Microvascular thrombosis is the least common variety of thrombosis but it can be seen in catastrophic APS (CAPS), which is a potentially lethal condition characterized by widespread microvascular thrombosis that leads to multiple organ failure.\(^2\) Diagnosis APS requires at least one of the clinical criteria and one of the laboratory criteria to be present (Table 1).

aPL antibodies

The term ‘aPL Abs’ encompasses antibodies that target protein antigens bound to anionic phospholipids and those binding anionic phospholipid antigens directly. In APS, predominant antibodies are directed against beta 2-glycoprotein I (β\(_2\)-GPI) and prothrombin. Less common antibodies are against phospholipids, i-e cardiolipin (CL), phosphotidylserine (PS), tissue plasminogen activator (tPA), plasmin, annexin A2(Ann A2) and thrombin.\(^3\)

β\(_2\)-glycoprotein I

β\(_2\)-glycoprotein I (β\(_2\)-GPI), also known as apolipoprotein H\(_2\), is a 50-kDa glycosylated protein having plasma concentration of 200 µg/mL (4µM).\(^4\) It is a member of complement control protein composed of 326 amino acids. It consists of 5 short consensus repeats of ≈60 amino acid residues, termed as domain I through domain V. The domain V has an extra 20-amino acid-long mobile tail and forms a binding site for negatively charged anionic phospholipids.\(^5\) Most aPL antibodies recognize domain I of β\(_2\)-GPI. In a healthy individual the free thiol form of β\(_2\)-GPI is characterized by a broken disulfide bridge at Cys32 and Cys60 and another at Cys288 and Cys326, predominates in the plasma. The disulfide bridges at these locations are broken by the oxidoreductases thioredoxin-1 and protein disulfide isomerase (PDI). Under conditions of oxidative stress, disulfide bonds form at these sites and oxidized form of β\(_2\)-GPI is immunogenic. This oxidized form of β\(_2\)-GPI predominates in patients with APS.\(^6\) (Figure 1)

Table 1: Diagnostic criteria of APS.\(^2\)

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<th>Clinical criteria</th>
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<td>1. Vascular thrombosis</td>
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<td>One or more clinical episodes of arterial, venous or small vessel thrombosis.</td>
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<td>2. Pregnancy morbidity</td>
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<td>(a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation.</td>
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<td>(b) One or more pre-term births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe pre-eclampsia or (ii) recognized features of placental insufficiency.</td>
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<td>(c) Three or more unexplained consecutive spontaneous miscarriages before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and maternal and paternal chromosomal causes excluded.</td>
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<td>1. Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart</td>
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<td>2. Anticardiolipin (aCL) antibody of immunoglobulin (Ig)G and/or IgM isotype in serum or plasma, present in medium or high titre (i.e. &gt;40GPL units or MPL units, or &gt; the 99th centile), on two or more occasions, at least 12 weeks apart.</td>
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<td>3. Anti-b2–glycoprotein I antibody of IgG and/or IgM isotype in serum or plasma (in titre &gt;the 99th centile), present on two or more occasions at least 12 weeks apart.</td>
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*APS is present if at least one of the clinical criteria and one of the laboratory criteria are met.
Conformations of β₂-GPI and binding of anti-β₂-GPI antibodies

Two conformations of β₂-GPI have been identified. One is circular inactive form of β₂-GPI that is present in the plasma and other is an elongated active form of β₂-GPI (Fig 2). In the circular form, the critical B-cell epitopes are hidden from immune system. Binding of β₂-GPI to anionic phospholipid surfaces results in the opening of β₂-GPI into a fishhook configuration, exposing domain I epitopes and allows binding of anti-β₂-GPI autoantibodies. Binding of anti-β₂-GPI antibodies to β₂-GPI leads to stabilization and dimerization of β₂-GPI that facilitates binding of β₂-GPI to several cell surface receptors and cell signaling (Fig 3).

Mechanisms predisposing to thrombosis

Mechanisms predisposing to thrombosis in APS are not very clear, however proposed pathogenic mechanism based on animal experiments are:

Triggers of thrombosis

The “two hit” model of thrombosis also exists in APS. According to this model an initiating “first hit” injury disrupts the endothelium and then a “second hit” potentiates thrombus formation. Experiments have shown that autoantibodies from patients with APS do not promote thrombus formation in the absence of vessel-wall injury. β₂-GPI does not bind unstimulated endothelium in vivo. Infections and recent surgery are recognized as precipitants of endothelial injury in CAPS. However, the initiating stimulus in most cases of thrombotic APS is not identified but it is postulated that disturbance of the redox balance in the vascular milieu in patients with APS may serve as a first hit that primes the endothelium, leading to formation of β₂-GPI immune complexes on the cell surface. Oxidative stress from exogenous sources, i.e., smoking may convert the vascular endothelial milieu into a prothrombotic phenotype. Oxidative stress can up-regulate the expression of Ann A2 and hence can play an important role in pathogenesis of APS.

Prothrombotic mechanisms involving the dysregulated activation of platelets, endothelial cells and monocytes by the anti-β₂-GPI Ab/β₂-GPI complex

Anti-β₂-GPI Ab/β₂-GPI complex induces intracellular signaling by binding to several cell surface receptors of platelets, endothelial cells and monocytes.

Platelets

On platelet surface, ApoER2 receptor and GPIbα subunit of the GPIb/IX/V receptor has been shown to bind β₂-GPI in vitro. The interaction of β₂-GPI to platelet surface receptors activates platelets, leading to thromboxane production and activation of the phosphoinositide-3 kinase/Akt pathway which further contributes to the activation of the α₃β₃ receptor on
platelets. aPL Abs also activate the p38MAPK/phospholipase A2 (PLA2) pathway, leading to the potentiation of thromboxane production by platelets and downstream activation of GPIba and ApoER2. Platelet activation leads to release of platelet factor 4 which facilitates dimerization of β2-GPI, further enhancing the formation of immune complexes on platelet surface.

**Endothelial cells** - Binding of β2-GPI to Ann A2 on endothelial cell surface leads to endothelial cell activation and expression of a procoagulant phenotype. Toll like receptors (TLRs), particularly TLR4 and TLR2, are present on endothelial cell surface and function as coreceptors for Ann A2 in aPL-mediated cell activation. Abs directed against Ann A2 itself have also been detected in 14.8-40.4% of patients with APS and these Abs are associated with in vitro prothrombotic changes including increased expression of TF and inhibition of tPA-mediated plasmin activation. aPL Abs can also activate nuclear factor-κB (NF-κB) pathway in endothelial cells leading to the expression of a procoagulant and proinflammatory phenotype. aPL Abs also increase expression of endothelial cell surface adhesion molecules, ie ICAM and E-selectin. Studies have shown that anti-β2-GPI autoantibodies antagonize the activity of endothelial nitric oxide synthase, resulting in decreased bioavailable nitric oxide, leading to monocyte adhesion to the endothelium.

**Monocytes** - β2-GPI colocalize with Ann A2 and TLR4 on the lipid rafts of monocytes. Anti-β2-GPI autoantibodies stimulate monocytes and increase tissue factor (TF) expression and release of tumor necrosis factor α (TNF-α) via induction of p38MAPK and NF-κB intracellular signaling pathways. (Figure 4)

**Prothrombotic mechanisms based on the disruption of protein C and antithrombin-III anticoagulant pathways**

Protein C (PC) pathway is an important endogenous anticoagulant pathway. Studies have shown that anti-β2-GPI Abs in the presence of β2-GPI inhibit activated PC (APC) anticoagulant activity in vitro. It is suggested that anti-β2-GPI Ab/β2-GPI complex may either compete with the components of APC complex for limited phospholipid binding sites or disrupt an interaction within the APC complex. aPL Abs can inhibit activity of PC directly or via its cofactor protein S. These Abs also bind to factor Va and VIIIa and protect them from proteolysis by APC. It is also demonstrated that some aPL Abs can cross react with heparin and heparinoid molecules and inhibit the acceleration of antithrombin-III activity by these molecules.

**Prothrombotic mechanisms based on the disruption of fibrinolysis**

Impairment of fibrinolysis by aPL may contribute to the development of thrombosis. Several studies have assessed the effects of aPL on the activity of the fibrinolytic system and several mechanisms are suggested for disruption of fibrinolysis in APS. Studies have reported that patients with APS have elevated levels of PAI-1 and decreased tPA, hence leading to impaired fibrinolysis. Plasma levels of lipoprotein a (Lpa) are also reported to be elevated in APS patients. Lpa has structural homology with plasminogen (PLG) and hence competes with PLG for binding to fibrin and interferes with plasmin-mediated fibrin degradation. Significant number of patients with APS have been reported to have antiplasmin antibodies and thus could block plasmin-mediated fibrinolysis. As discussed previously, Ann A2 antibodies have been demonstrated in patients with APS and these antibodies block Ann A2 ability to promote tPA-mediated PLG activation. Furthermore anti-β2-GPI Abs interfere with the interaction of β2-GPI with tPA and enhancement of tPA activity. (Figure 5)

**Complement activation in APS**

In vivo murine models have shown role of activation of classical complement pathway in thrombosis associated with APS. Complement activation by aPL Abs generate C5a, which binds and activates neutrophils, leading to tissue factor expression.

**Pathogenesis of obstetrical complications in APS**

Several mechanisms have been suggested for pregnancy failure in APS. Initially intraplacental thrombosis was suggested as main pathogenic mechanism responsible for pregnancy failure but this is not specific for APS and can be seen associated with other conditions as well. There is strong evidence of disruption of annexin V in the placental circulation in APS patients. Annexin V covers the thrombogenic anionic placental surfaces and prevents the activation of the coagulation cascade by inhibiting the binding of activated coagulation factors in vivo. Expression of annexin V is found to be markedly reduced in aPL syndrome placentas as compared to controls and it is hypothesized that aPL
Abs interfere with the formation of antithrombotic annexin V shield leading to pregnancy failure. Disruption of trophoblast function is described as a pathogenic mechanism of early pregnancy loss in APS. Trophoblasts can express antigens such as PS and β2-GPI, which can be potentially targeted by the relevant aPL Abs. In vitro studies have shown that interaction of aPL Abs with β2-GPI on trophoblast surface results in inhibition of trophoblast invasion and gonadotropin secretion. Anti-β2-GPI Abs also cause trophoblasts to express a proinflammatory cytokine profile via activation of the TLR4/myeloid differentiation primary response protein 88 (MyD88) pathway characterized by increased IL-8, IL-1β, MCP-1 and growth-regulated oncogene-α resulting in increased trophoblast apoptosis. Complement activation may also contribute to recurrent fetal loss in APS.

Figure 5: Dysregulation of anticoagulant and fibrinolytic systems in APS.

References