Mechanisms of Disease

The Pathogenesis of the Antiphospholipid Syndrome

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The antiphospholipid syndrome is a prothrombotic disorder that can affect both the venous and arterial circulations.\(^1,2\) The deep veins of the lower limbs and the cerebral arterial circulation are the most common sites of venous and arterial thrombosis, respectively.\(^2\) However, any tissue or organ vascular bed can be affected. Catastrophic antiphospholipid syndrome, which is characterized by clots in multiple small vascular beds and leads to multiorgan failure with high mortality, develops in a small subgroup of patients.\(^2,3\) In situations in which histopathological confirmation is sought, thrombosis should be present without evidence of inflammation in the vessel wall.\(^1\)

The other major clinical manifestations of the antiphospholipid syndrome are obstetrical. They include the unexplained death of one or more morphologically normal fetuses at or beyond the 10th week of gestation, the premature birth of one or more morphologically normal neonates before the 34th week of gestation because of either eclampsia or severe preeclampsia, and three or more unexplained, consecutive spontaneous abortions before the 10th week of gestation.\(^1\)

The revised classification criteria for the antiphospholipid syndrome (2006) emphasize the presence of specific autoantibodies as an essential component of the diagnosis.\(^1\) The persistence (for >12 weeks) of high titers of autoantibodies of the IgG or IgM isotype, detected by enzyme-linked immunosorbent assay (ELISA) for anti-β\(_2\)-glycoprotein I or anticardiolipin antibodies or by lupus-anticoagulant assays, is required.\(^1\) The lupus-anticoagulant assays detect autoantibodies that have the ability to prolong clotting time in vitro. Such antibodies target β\(_2\)-glycoprotein I and prothrombin, both of which are plasma proteins that bind to anionic phospholipids.\(^4-6\) The term “antiphospholipid antibodies” is often used to encompass any or all of the antibodies detected by ELISA and the lupus-anticoagulant assays. A diagnosis of the antiphospholipid syndrome is made if at least one of the above clinical criteria and one of the laboratory criteria are met.\(^1\)

Role of Autoantibodies with Lupus-Anticoagulant Activity

A positive test for lupus anticoagulant is a stronger risk factor for thrombosis and adverse pregnancy outcomes after 12 weeks of gestation than positivity for either anti-β\(_2\)-glycoprotein I or anticardiolipin antibodies.\(^7-9\) A case–control study designed to estimate the contribution of genetic and acquired risk factors to a first episode of venous thrombosis in the general population of persons younger than 70 years of age (with no known cancer) showed that 3.1% of persons with venous thrombosis were positive for lupus anticoagulant, as compared with 0.9% of controls (odds
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In a case–control study focusing on risk factors for stroke in women in the general population younger than 50 years of age, 17% of the patients with stroke were positive for lupus anticoagulant, as compared with 0.7% of controls (odds ratio, 43.1). The risk was further increased by taking oral contraceptive pills (odds ratio, 201.0) or smoking (odds ratio, 87.0). Approximately 1% of women trying to get pregnant have recurrent miscarriages; of these women, approximately 10 to 15% are estimated to have obstetrical antiphospholipid syndrome. Positivity for lupus anticoagulant is the strongest predictor of subsequent thrombosis in purely obstetrical antiphospholipid syndrome; the annual incidence of deep-vein thrombosis is 1.46%, and the annual incidence of stroke is 0.32%.

Lupus anticoagulant due to anti–β₂-glycoprotein I autoantibodies correlates more strongly with a risk of thrombosis than does lupus anticoagulant due to antiprothrombin autoantibodies. The risk of a first thrombotic event among asymptomatic persons who are positive for lupus anticoagulant, anticardiolipin antibodies, and anti–β₂-glycoprotein I antibodies — so-called triple-positive patients — is 5.3% per year. These patients have high titers of autoantibodies that bind the major B-cell epitope on domain I of the β₂-glycoprotein I molecule. Domain I anti–β₂-glycoprotein I autoantibodies confer lupus-anticoagulant activity associated with the highest risk of thrombosis. Assays that detect autoantibodies to the phosphatidylserine–prothrombin complex (in contrast to prothrombin alone) may help establish the diagnosis of the antiphospholipid syndrome and the associated level of risk, in conjunction with the lupus-anticoagulant assays and the ELISA for anti–β₂-glycoprotein I antibodies. The usefulness of also performing the ELISA for anticardiolipin antibodies to diagnose thrombotic antiphospholipid syndrome is being debated.

Autoantibodies from patients with the antiphospholipid syndrome potentiate thrombus formation when infused into mice in which the blood vessel has been injured. The thrombogenic properties are eliminated when the fraction of anti–β₂-glycoprotein I autoantibodies is removed. We review the mechanisms that may contribute to thrombosis in the syndrome, integrating clinical biomarker data, results of in vitro mechanistic studies, and relevant animal models (Table 1).
Thrombotic Mechanisms

Post-Translational Redox Modifications of \( \beta_2 \)-Glycoprotein I

A number of findings suggest that the antiphospholipid syndrome is characterized by increased oxidative stress. Paraoxonase activity, which accounts for the antioxidant properties of high-density lipoprotein cholesterol (preventing oxidation of low-density lipoprotein [LDL] cholesterol), is significantly decreased in persons with the syndrome,\textsuperscript{26,27} whereas levels of 8-epi-prostaglandin \( F \textsubscript{2\alpha} \), a biomarker of lipid peroxidation, are elevated.\textsuperscript{28} Plasma levels of \( \beta_2 \)-glycoprotein I–oxidized LDL complexes are elevated in persons with the antiphospholipid syndrome as compared with healthy controls.\textsuperscript{72}

Oxidative stress plays a direct role in the structure and function of \( \beta_2 \)-glycoprotein I in patients with the antiphospholipid syndrome. Purified \( \beta_2 \)-glycoprotein I is composed of four domains (domains I through IV) that contain two disulfide bridges each and a fifth domain (domain V) that contains an extra disulfide bridge linking cysteine (Cys) 288 with Cys326\textsuperscript{73} (Fig. 1). In healthy persons, the free thiol form of \( \beta_2 \)-glycoprotein I predominates in the plasma, characterized by a broken disulfide bridge at Cys32 and Cys60 and another at Cys288 and Cys326.\textsuperscript{25,30,74,75} The former pair of free thiols are near the antiphospholipid syndrome B-cell epitope in domain I,\textsuperscript{16,17} and the latter are near the T-cell epitope in domain V.\textsuperscript{76} The disulfide bridges at these locations are broken by the oxidoreductases thioredoxin-1 and protein disulfide isomerase (PDI).\textsuperscript{74,75} Under conditions of oxidative stress, disulfide bonds form at these sites.\textsuperscript{25} The relative proportion of plasma \( \beta_2 \)-glycoprotein I in the oxidized versus the free thiol form was significantly greater in patients with the antiphospholipid syndrome than in patients with autoimmune disease with or without persistent antiphospholipid antibodies but without the antiphospholipid syndrome, patients with vascular thrombosis without antiphospholipid antibodies, and healthy volunteers (\( P<0.001 \) for all comparisons).\textsuperscript{25} Patients with the syndrome who were positive for both lupus anticoagulant and anti–\( \beta_2 \)-glycoprotein I antibodies had significantly higher levels of oxidized \( \beta_2 \)-glycoprotein I than patients who were positive for anti–\( \beta_2 \)-glycoprotein I antibodies alone.\textsuperscript{25}

Supporting the notion that oxidation unmasks the critical antiphospholipid syndrome B-cell epitope, anti–\( \beta_2 \)-glycoprotein I antibodies purified from mice and rabbits that had been immunized with \( \beta_2 \)-glycoprotein I displayed decreased binding to oxidoreductase-treated \( \beta_2 \)-glycoprotein I,\textsuperscript{74} as did autoantibodies that were affinity-purified from patients with the antiphospholipid syndrome (Fig. 2).

Conformations of \( \beta_2 \)-Glycoprotein I

\( \beta_2 \)-glycoprotein I can potentially exist in a circular form, with domain I interacting with domain V.\textsuperscript{77} In this form, the critical B-cell epitope is hidden from the immune system.\textsuperscript{77} On binding to an anionic phospholipid surface through domain V, the circular form of \( \beta_2 \)-glycoprotein I opens up to a fishhook configuration, exposing the domain I epitope and allowing domain I anti–\( \beta_2 \)-glycoprotein I autoantibodies to bind.\textsuperscript{77} The presence of the circular form has yet to be directly shown in...
human plasma; however, circumstantial evidence points to its in vivo presence. Domain I anti–β2-glycoprotein I autoantibodies were induced in mice in which protein H (derived from Streptococcus pyogenes) was administered.78 Protein H changes the conformation of β2-glycoprotein I from the circular to the theoretically more immunogenic open form in vitro.78 The relationship of the circular form of β2-glycoprotein I to the free thiol form has not been determined.

TRIGGERS OF THROMBOSIS

The “two hit” model of thrombosis associated with the antiphospholipid syndrome proposes that an initiating “first hit” injury disrupts the endothelium, and a “second hit” potentiates thrombus formation.79 Autoantibodies from patients with the antiphospholipid syndrome that are infused into mice do not promote thrombus formation in the absence of vessel-wall injury.23,24 A key step in allowing β2-glycoprotein I immune complexes to form on the cell surface is endothelial-cell priming.23 β2-glycoprotein I does not bind unstimulated endothelium in vivo.80 In catastrophic antiphospholipid syndrome, infection and recent surgery are recognized precipitants of endothelial injury.81 However, the initiating stimulus is not identified in most cases of thrombotic antiphospholipid syndrome. We postulate that disturbance of the redox balance in the vascular milieu in patients with the antiphospholipid syndrome may constitute a substantial first hit that primes the endothelium, allowing β2-glycoprotein I immune complexes to form on the cell surface and assert their pathogenicity.

Patients with the antiphospholipid syndrome have significantly lower levels of the endothelium-protective free thiol form of β2-glycoprotein I that provides a buffer against oxidative stress than do healthy persons.25,30 The odds ratio for reduced levels of the free thiol form of β2-glycoprotein I in patients with the antiphospholipid syndrome, as compared with age-matched and sex-matched controls, was reported to be 4.1 (95% confidence interval [CI], 1.9 to 8.8).25 Oxidative stress from exogenous sources such as smoking82 may tip the vascular endothelial milieu toward a prothrombotic phenotype. For example, oxidative stress can up-regulate the expression of annexin A2,83 an endothelial cell-surface receptor for β2-glycoprotein I that has an important role in the pathogenesis of the antiphospholipid syndrome.42 Among young women, the odds ratio for ischemic stroke in the presence of lupus anticoagulant is 43.1, and it increases to 87.0 with concurrent oxidative stress (and other pathophysiological disturbances) induced by smoking.8 In a murine model of thrombosis, reactive oxygen species induced platelet aggregation, endothelial-cell stimulation, and expression of von Willebrand factor.31 N-acetylcysteine (NAC), a scavenger of reactive oxygen species, inhibited thrombus formation in this model.31 The therapeutic value of NAC in the antiphospholipid syndrome may be worth exploring (Fig. 3).

ENDOTHelial NITRIC oxide SYNTHASE

Patients with the antiphospholipid syndrome have decreased levels of plasma nitrite, as compared with controls.32 They also have impaired endothelium-dependent vascular responses,27 which suggests that the activity of endothelial nitric oxide synthase is abnormal.

Endothelium-derived nitric oxide plays an im-

Figure 2. Results of Direct Enzyme-Linked Immunosorbent Assays of Affinity-Purified Antiphospholipid Antibodies in Patients with the Antiphospholipid Syndrome.

The oxidized (S–S) form or free thiol (SH) form of β2GPI was coated onto microtiter plates, and antibodies from individual patients were assessed for binding. Symbols denote individual antibody preparations. Increased optical density denotes an increase in anti-β2GPI binding to oxidized β2GPI.
important role in healthy endothelial function.\textsuperscript{84-86} It is produced by enzymatic conversion of \textit{L}-arginine by endothelial nitric oxide synthase.\textsuperscript{87} Reduced expression and activity of endothelial nitric oxide synthase can result in the generation of superoxide and peroxynitrite.\textsuperscript{88} Because nitric oxide has an exceptionally short half-life, the activity of endothelial nitric oxide synthase is estimated by measuring nitric oxide metabolites in plasma. Plasma nitrite most closely reflects changes in the activity of endothelial nitric oxide synthase in humans.\textsuperscript{89}

In a murine model, domain I anti–\(\beta_2\)-glycoprotein I autoantibodies decreased bioavailable nitric oxide by antagonizing the activity of endothelial nitric oxide synthase, which led to monocye adhesion to the endothelium.\textsuperscript{33} The autoantibodies exerted their pathogenic effects in this model in a manner that was independent of complement and Fc receptor.\textsuperscript{33} Endothelial nitric oxide–dependent arterial relaxation was inhibited by domain I anti–\(\beta_2\)-glycoprotein I autoantibodies in these mice,\textsuperscript{33} reflecting vascular disturbances analogous to those in humans with antiprophospholipid autoantibodies.\textsuperscript{27} Inhibition of the activity of endothelial nitric oxide synthase was mediated by the F(ab\textsuperscript{\prime})\textsubscript{2} portion of domain I anti–\(\beta_2\)-glycoprotein I autoantibodies, which dimerized \(\beta_2\)-glycoprotein I molecules attached to apolipoprotein E (ApoE) receptor 2 (LDL receptor–related protein 8), cross-linking and activating ApoE receptor 2.\textsuperscript{33} Anti–\(\beta_2\)-glycoprotein I autoantibodies did not enhance leukocyte adhesion to the endothelium, nor did they potentiate in vivo thrombus formation in mice deficient in endothelial nitric oxide synthase or ApoE receptor 2, findings that indicate the critical role of these receptors in pathogenicity mediated by anti–\(\beta_2\)-glycoprotein I autoantibodies.\textsuperscript{33}
glutaryl–coenzyme A (HMG-CoA) reductase — block the thrombogenic properties of antiphospholipid autoantibodies in vitro and in vivo. Statins may be protective in the antiphospholipid syndrome owing in part to their up-regulation of endothelial nitric oxide synthase.

A number of strategies are being pursued to disrupt the formation of β₂-glycoprotein I immune complexes on cell surfaces. Domain V of β₂-glycoprotein I binds the A1 ligand–binding type A module of ApoE receptor 2. A dimeric A1–A1 molecule blocks the formation of β₂-glycoprotein I immune complexes on anionic phospholipid surfaces. Mice treated with soluble monomeric A1 are protected from the thrombogenic effects of anti–β₂-glycoprotein I autoantibodies, providing proof of principle that A1–A1 dimers have therapeutic value (Fig. 3). Infusion of synthetic domain I of β₂-glycoprotein I was protective in a murine model of thrombosis induced by anti–β₂-glycoprotein I autoantibodies (Fig. 3).

The binding of pathogenic domain I anti–β₂-glycoprotein I autoantibodies to β₂-glycoprotein I may be inhibited by breaking the disulfide bond and inducing free thiol formation at Cys32 and Cys60 within domain I of β₂-glycoprotein I in vitro (Fig. 3). NAC is able to break an analogous disulfide bond within von Willebrand factor, with implications for treating patients with thrombotic thrombocytopenic purpura, and exploration of its therapeutic potential in the antiphospholipid syndrome may be of value (Fig. 3).

**ENDOTHELIAL CELLS AND MONOCYTES**

Antiphospholipid autoantibodies may up-regulate the cell-surface expression of proadhesive and procoagulant molecules such as tissue factor. Anti–β₂-glycoprotein I autoantibodies may induce signaling by means of a multiprotein complex on the endothelial cell surface that includes annexin A2 (bound by β₂-glycoprotein I), toll-like receptor 4 (TLR4), calreticulin, and nucleolin. Intracellular activation downstream of TLR4 occurs through myeloid differentiation factor 88, culminating in activation of nuclear factor κB (NF-κB). The targeting of NF-κB is a therapeutic option. The absence of annexin A2 was reported to protect mice against the prothrombotic effects of infused autoantibodies from patients with the antiphospholipid syndrome. Mouse resistance to lipopolysaccharide was also protected, a finding that supports the relevance of TLR4 in the pathogenesis of the antiphospholipid syndrome.

β₂-glycoprotein I has been shown to colocalize with annexin A2 and TLR4 on the lipid rafts of monocytes. Anti–β₂-glycoprotein I autoantibodies stimulate monocytes to increase tissue factor expression and release tumor necrosis factor α (TNF-α). Antiphospholipid autoantibody–induced tissue factor expression is mediated through a number of intracellular signaling pathways.

In one study, 19 of 32 affinity-purified antibodies from patients with the antiphospholipid syndrome were shown to induce activation of human monocytes and endothelial cells. However, in that series of experiments, the results showed that activation occurred through toll-like receptor 2 (TLR2) and CD14, not TLR4. Further work is needed to resolve these discrepancies.

Autoantibodies from patients with the antiphospholipid syndrome can disrupt the mitochondrial function of monocytes and neutrophils, leading to the generation of various intracellular reactive oxygen species and the subsequent expression of tissue factor. Antibodies from patients with the syndrome do not colocalize with mitochondria, suggesting that mitochondrial dysfunction is induced through undefined indirect pathways. In one study, the inhibition of intracellular reactive oxygen species in monocytes with the use of NAC, vitamin C, or mitochondrial cofactor coenzyme Q₁₀ prevented the up-regulation of tissue factor induced by antiphospholipid autoantibodies (Fig. 3).

Human monocytes are activated in a distinct manner by polyclonal autoantibodies derived from patients with purely thrombotic antiphospholipid syndrome as compared with monocyte activation by autoantibodies from patients with obstetrical antiphospholipid syndrome. Autoantibodies from patients with thrombotic antiphospholipid syndrome induce tissue factor expression, which is caused by the autoantibody fraction that binds β₂-glycoprotein I.

**TISSUE FACTOR**

Tissue factor is the key initiator of the extrinsic coagulation pathway. It is located on cell surfaces and microparticles in an encrypted, inactive form. On vessel injury leading to exposure...
of phosphatidylserine, tissue factor becomes de-encrypted and activated, enabling it to bind factor VIIa, which leads to activation of factor IX and factor X. The relevance of tissue factor in the pathogenesis of the antiphospholipid syndrome is supported by the results of in vitro and in vivo murine studies. Thiols exchange reactions play an important role in the regulation of tissue factor from the encrypted to the de-encrypted form. PDI, an extracellular regulator of thiol exchange, is associated with cell-surface tissue factor and is required for tissue factor-dependent thrombosis in vivo. Hence, PDI inhibitors may therapeutically target tissue factor in the pathogenesis of the antiphospholipid syndrome (Fig. 3).

**Factor XI**

Elevated levels of coagulation factor XI confer a predisposition to venous thrombosis and stroke in the general population, mirroring the distribution of thrombosis in patients with the antiphospholipid syndrome. A link has been discovered between the antiphospholipid syndrome and dysregulated activation of factor XI. Factor XI is a proenzyme that is cleaved to its active form (factor XIa) by factor XIIa or thrombin. Factor XIa is responsible for factor IX activation, ultimately leading to a burst of thrombin generation. Factor XI can be a substrate of the oxidoreductases thioredoxin 1 and PDI, which target the factor XI intrachain disulfide bonds at Cys118–Cys147 and Cys362–Cys482 and the intact disulfide-bridge form of factor XI are found in human plasma. Both the free thiol form and the intact disulfide-bridge form of factor XI are associated with increased risk of bleeding in these models, making factor XI an attractive therapeutic target.

The Cys362–Cys482 free thiols may not be critical for the potentiation of factor XI activation. Mutagenesis of Cys362 and Cys482 to alanine, which eliminates the disulfide bridge between the heavy chain and light chain of factor XI, leads to decreased ligation of factor IX by the factor XIa mutants.

**Platelets**

β2-glycoprotein I can interact with the von Willebrand factor receptor glycoprotein Ibα and ApoE receptor 2. This enables anti–β2-glycoprotein I autoantibodies to cross-link these receptors, leading to potentiation of platelet activation, the release of thrombospondin A2, and an increase in platelet adhesiveness. Platelet factor 4, a cationic protein released by activated platelets, can facilitate the dimerization of β2-glycoprotein I, enhancing the formation of pathogenic immune complexes on the platelet surface.

**Annexin A5 Anticoagulant Shield and Hydroxychloroquine**

In one model of the pathogenesis of the antiphospholipid syndrome, annexin A5 binds to phosphatidylerine surfaces, forming a shield that inhibits the formation of procoagulant complexes. An in vitro study has shown that domain I anti–β2-glycoprotein I autoantibodies in complex with β2-glycoprotein I can disrupt the shield, exposing procoagulant phosphatidylerine and hence predisposing to thrombosis. Hydroxychloroquine inhibits the ability of β2-glycoprotein I immune complexes to disrupt the annexin A5 matrix on the endothelial-cell surface (Fig. 3). Hydroxychloroquine diminished antiphospholipid autoantibody–mediated thrombosis in vivo in a murine model.

**Complement and Neutrophils**

Case reports document the use of the C5 inhibitor eculizumab to prevent antiphospholipid syndrome–associated thrombotic microangiopathy that complicates renal transplantation, and to treat patients with acute catastrophic antiphospholipid syndrome. In vivo murine studies implicating the activation of the classical complement pathway in thrombosis associated with the antiphospholipid syndrome were the basis for the use of eculizumab in these case reports. Activation of complement by antiphospholipid autoantibodies generates C5a, which binds and activates neutrophils, leading to tissue...
factor expression. On the basis of murine studies, C3 and C5 have been proposed as possible therapeutic targets for treating obstetrical antiphospholipid syndrome (Fig. 3).

**DISTURBANCE OF INNATE IMMUNITY**

The prevalence of lupus-anticoagulant positivity among patients with systemic lupus erythematosus (SLE) is 30%, and the presence of lupus-anticoagulant positivity in such patients is associated with an increased risk of thrombosis (odds ratio, 5.6). Forty percent of patients with the antiphospholipid syndrome also have SLE, and 37% of patients with SLE have anti-β2-glycoprotein I autoantibodies. These findings suggest that there is overlap in the pathogenesis of SLE and that of the antiphospholipid syndrome.

A relationship between the two disorders is supported by the spontaneous development of domain I anti-β2-glycoprotein I autoantibodies and the development of a syndrome analogous to human antiphospholipid syndrome in a murine model of lupus, NZW x BXSB F1 male mice. In this model, a major contributor to pathogenesis is TLR7 duplication resulting from translocation of TLR7 from the X to the Y chromosome in the BXSB male mice. Dysregulated activation of TLR7 in plasmacytoid dendritic cells by RNA containing immune complexes and the generation of autoantibodies (e.g., anti-Sm and anti-RNP antibodies) create a positive-feedback loop for further autoantibody generation. Antibodies from patients with the antiphospholipid syndrome are able to up-regulate the expression of TLR7 and TLR8 in plasmacytoid dendritic cells and monocytes, respectively, as well as their translocation from the endoplasmic reticulum to the endosome, sensitizing the cells to TLR7 and TLR8 ligands. These effects depend on the uptake of antiphospholipid autoantibodies into the endosome, the activation of NADPH oxidase, and the generation of superoxide. Inhibition of TLR7 and TLR8 may be an attractive therapeutic target in patients with SLE and antiphospholipid autoantibodies. In keeping with this idea, hydroxychloroquine has been shown to inhibit TLR7 and TLR8 ligands (Fig. 3), and it is associated with a reduced odds ratio for the persistence of antiphospholipid autoantibodies in patients with SLE.

B-cell activating factor (BAFF) is a cytokine that is crucial for B-cell survival. The BAFF-inhibiting antibody belimumab has recently been approved for the treatment of SLE. BAFF inhibition prevented thrombosis in NZW x BXSB F1 male mice, a finding that suggests it may have a role in the prevention of thrombosis associated with the antiphospholipid syndrome in high-risk patients with SLE (Fig. 3).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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