



# Pathophysiology of Antiphospholipid Syndrome

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## Abstract

The antiphospholipid syndrome is characterized by antibodies directed against phospholipid-binding proteins and phospholipids attached to cell membrane receptors, mitochondria, oxidized lipoproteins, and activated complement components. When antibodies bind to these complex antigens, cells are activated and the coagulation and complement cascades are triggered, culminating in thrombotic events and pregnancy morbidity that further define the syndrome. The phospholipid-binding proteins most often involved are annexins II and V,  $\beta_2$ -glycoprotein I, prothrombin, and cardiolipin. A distinguishing feature of the antiphospholipid syndrome is the “lupus anticoagulant.” This is not a single entity but rather a family of antibodies directed against complex antigens consisting of  $\beta_2$ -glycoprotein I and/or prothrombin bound to an anionic phospholipid. Although these antibodies prolong in vitro clotting times by competing with clotting factors for phospholipid binding sites, they are not associated with clinical bleeding. Rather, they are thrombogenic because they augment thrombin production in vivo by concentrating prothrombin on phospholipid surfaces. Other antiphospholipid antibodies decrease the clot-inhibitory properties of the endothelium and enhance platelet adherence and aggregation. Some are atherogenic because they increase lipid peroxidation by reducing paraoxonase activity, and others impair fetal nutrition by diminishing placental antithrombotic and fibrinolytic activity. This plethora of destructive autoantibodies is currently managed with immunomodulatory agents, but new approaches to treatment might include vaccines against specific autoantigens, blocking the antibodies generated by exposure to cytoplasmic DNA, and selective targeting of aberrant B-cells to reduce or eliminate autoantibody production.

## Keywords

- ▶ antiphospholipid antibodies
- ▶ phospholipid-binding proteins
- ▶ lupus anticoagulant

## Introduction

In 1983 Hughes<sup>1</sup> described a syndrome that included arterial and venous thromboses, strokes, and obstetrical disorders, and was associated with an antilipid antibody, the lupus anticoagulant (LAC). Despite its name, the LAC was observed to be a strong risk factor for thrombosis rather than bleeding, but why it behaved in this fashion was unclear. During the past 40 years, the LAC was associated with anti-phosphati-

dyserine/prothrombin (anti-PS/PT) antibodies,<sup>2</sup> and a variety of other autoantibodies were identified that are directed against complexes of phospholipid,  $\beta_2$ -glycoprotein I ( $\beta_2$ -GPI), and other phospholipid-binding proteins.<sup>3,4</sup> LAC and other autoantibodies that bind to phospholipid-binding proteins are designated antiphospholipid antibodies (APAs), and contribute to the distinctive pathologic features of the antiphospholipid syndrome (APS). In this review, the several phospholipid-binding proteins are described, and the

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cellular receptors and other tissues that bind them are identified. Autoantibodies target these complexes and trigger pathologic processes that bring about thrombosis, premature atherosclerosis, and pregnancy morbidity.

APS is classified as primary (no underlying disorder) or secondary (to infection, neoplasm, or other autoimmune disease). In a series of 100 patients with LAC, Triplett et al<sup>5</sup> reported that 34% were drug-associated (chlorpromazine, quinidine, phenytoin, procainamide), 13% autoimmune, 10% infections, and 43% miscellaneous. Greaves<sup>6</sup> classifies APS as secondary if it occurs in association with systemic lupus erythematosus (SLE) or other connective tissue disorder, and primary if there is no underlying disorder. Campbell et al<sup>7</sup> distinguish anticardiolipin antibodies (ACAs) from individuals with primary APS from ACA in patients with syphilis; the former is specific for PS and enhances agonist-induced platelet activation and aggregation. Although APAs are present in 62% of patients with syphilis, leprosy, and human immunodeficiency virus infection, autoantibodies to tissue factor pathway inhibitor (anti-TFPI) are observed in  $\leq 10\%$  versus 38% in those with primary APS.<sup>8,9</sup> Fewer thrombotic complications might be anticipated because thrombogenic autoantibodies are infrequent in secondary APS, but recent experience with coronavirus disease 2019 (Covid-19) suggests this is not always the case.

### Antiphospholipid Syndrome Secondary to Covid-19 Infection

In April 2020, Zhang et al<sup>10</sup> reported cerebral infarcts and antibodies to anti- $\beta_2$ -GPI and cardiolipin in three patients, and Harzallah et al<sup>11</sup> detected LAC in 45% of 56 patients with Covid-19 infection. Another study found 31 of 34 patients had LAC, and the factor XII level was less than 50IU/dL in 7% of 216 patients.<sup>12</sup> Decreased factor XII has been observed previously in 20.9% of patients with LAC.<sup>13</sup> An examination of serum samples from 172 hospitalized coronavirus patients reported high-titer APAs in 30%; most were immunoglobulin M and directed against cardiolipin in 7.6%,  $\beta_2$ -GPI in 4.1%, and PS/PT in 12%.<sup>14</sup> Higher APA titers were associated with higher platelet counts, the release of more neutrophil extracellular traps (NETS), and more severe respiratory disease; injection of the antibodies into mice accelerated venous thrombosis. The incidence of confirmed venous thromboembolism in hospitalized Covid-19 patients is 4.8% and total thrombotic complica-

tions 9.5%,<sup>15</sup> but in those requiring intensive care, thrombosis rates can be as high as 31% and correlate with evidence of antibody-induced platelet PS externalization and apoptosis.<sup>16,17</sup> Autopsy data reveal megakaryocytes and platelet-fibrin thrombi in the lungs, heart, and kidneys.<sup>18</sup> However, major thrombotic events are not associated with the APA, and the  $\beta_2$ -GPI epitopes targeted by the antibodies differ from those observed in patients with APS.<sup>19</sup> Although the high incidence of thrombosis appears to be related to the presence of APA, other factors associated with severe inflammation such as cytokines, complement factors, and NETS might be contributory.<sup>20</sup> There appears to be little distinction between primary and secondary APS when clinical outcomes (thrombosis, strokes, organ damage) are considered.

## Phospholipid-Binding Proteins

### Annexins

Annexins are proteins consisting of four repetitive domains of approximately 70 amino acids each that participate in  $\text{Ca}^{2+}$ -mediated binding to negatively charged phospholipids (**► Table 1**). Annexin II mediates the assembly of plasminogen and tissue plasminogen activator (t-PA) on cell membranes, enhancing tissue-based fibrinolysis.<sup>21</sup>  $\beta_2$ -GPI binds to annexin II on the endothelial cell surface. In people susceptible to the APS, the  $\beta_2$ -GPI-annexin II complex might stimulate anti- $\beta_2$ -GPI antibody formation. Activation of their endothelial cells occurs when the anti- $\beta_2$ -GPI antibodies cross-link  $\beta_2$ -GPI bound to annexin II.<sup>22</sup> These antibodies are thrombogenic because they not only inhibit surface plasmin expression but also stimulate the release of tissue factor.<sup>23</sup>

Annexin V functions as an anticoagulant by forming a crystalline shield over the exposed anionic phospholipids of injured cell membranes, preventing the formation of activated clotting factor complexes (the tenase and prothrombinase complexes).<sup>24</sup> This annexin shield is disrupted by APA bound to epitope G40-R43 on domain I of  $\beta_2$ -GPI.<sup>25</sup> Circulating apoptotic endothelial cells bearing annexin V are increased in young women with SLE, and are associated with elevated levels of tissue factor.<sup>26</sup> Loss of the annexin V shield might enable coagulation complexes to bind to the membrane phospholipids of placental trophoblasts, initiate thrombus formation, and adversely affect fetal nutrition.<sup>27</sup>

**Table 1** Major phospholipid (PL)-binding proteins

Protein	Size	Location	Description
Annexins	36 kd, 70 aa, repeats in $\alpha$ – helix	II: cell granules, membranes, rafts V: placenta	$\text{Ca}^{2+}$ -dependent PL binding; II binds S100A10, t-PA
$\beta_2$ -Glycoprotein I	48 kd, 326 aa	Plasma: 200 $\mu\text{g}/\text{mL}$	Multimer; circular form assumes J-shape when bound to PL
Cardiolipin	1,466 g/mol	Mitochondrial inner membrane	Diphosphatidyl glycerol; structural integrity of respiratory chain
Vimentin	310 aa-polymerizes	Skin and other organs; cell surface and extracellular matrix	Phosphorylated filamentous protein

Abbreviations: aa, amino acids; t-PA, tissue plasminogen activator.

### $\beta_2$ -Glycoprotein I

$\beta_2$ -GPI is a 48-kDa plasma protein composed of 326 amino acid residues deployed in five domains; it forms a circular structure in plasma when domain I interacts with domain V. Binding of the positively charged lysine cluster on domain V to negatively charged phospholipids extends the molecule into a fishhook configuration, exposing cryptic epitopes in domain I.<sup>28</sup> Immunogenicity is attributed to exposure of these epitopes as well as oxidation of the terminal sulfhydryl groups of  $\beta_2$ -GPI.<sup>4</sup> The developing antibodies target various domains of  $\beta_2$ -GPI; those directed against a domain I epitope comprising Lys39 and Arg43 have LAC activity.<sup>29</sup> This is because these  $\beta_2$ -GPI-antibody complexes can directly interact with factor V, attenuating its activation by factor Xa.<sup>30</sup>

$\beta_2$ -GPI is an antibacterial plasma protein with several functions related to hemostasis; these include augmenting phagocytosis of phospholipid-exposing microparticles and apoptotic cells, inhibition of platelet adhesion and aggregation mediated by von Willebrand factor (VWF) and adenosine diphosphate, and prevention of inactivation of protein S by C4b-binding protein.<sup>31,32</sup> The antithrombotic functions of  $\beta_2$ -GPI are impaired by the development of antibodies to the protein. Furthermore,  $\beta_2$ -GPI-antibody complexes bind to cellular receptors on endothelial cells, monocytes, neutrophils, and platelets, activating these cells and enhancing their thrombogenicity.

### Cardiolipin

Cardiolipin is an anionic phospholipid containing four unsaturated fatty acids, and is chiefly located on the inner mitochondrial membrane of the heart. It is a common target for antibodies (ACAs) that occasionally cross-react with other negatively charged phospholipids. ACAs are present in 44% and LAC in 34% of patients with SLE, and both are prevalent in various non-SLE disorders.<sup>33</sup> ACA, measured by immunoassay, is closely correlated with LAC as assessed by prolongation of the activated partial thromboplastin time ( $r = 0.7$ ).<sup>34</sup>

### Vimentin/Cardiolipin Complexes

Patients with clinical features suggesting the presence of APA but with negative tests for LAC, ACA, and anti- $\beta_2$ -GPI might have antibodies to a complex of vimentin and cardiolipin.<sup>35</sup> Vimentin is an endothelial cell phospholipid-binding protein that has an affinity for cardiolipin. Anti-vimentin/cardiolipin antibodies induce phosphorylation of interleukin (IL)-1 receptor-associated kinase, leading to production of nuclear factor-kappa B (NF- $\kappa$ B). APAs incubated with cultured endothelial cells stimulate the expression of tissue factor, E-selectin, and inducible nitric oxide synthase, probably by phosphorylation of p38 MAPK and activation of NF- $\kappa$ B.<sup>36,37</sup>

### The Antibodies and Their Targets

APS antibodies attack cells, cellular receptors, and hemostatic proteins either alone or in complexes with phospholipid-binding proteins; some APA targets are described in **Table 2**. It has been proposed that in disorders such as

SLE, anionic phospholipids on apoptotic cell surfaces provide binding sites for plasma proteins, exposing neo-epitopes that provoke APA.<sup>38</sup> The antibodies might indicate the presence of circulating apoptotic cells, which could account for the elevated risk of thrombosis in patients with APS.

### Cells and Cellular Receptors

#### Endothelial Cells

The endothelium releases a variety of factors that retard thrombosis, but its antithrombotic activity is severely compromised by APA. For example, the endothelial protein C receptor (EPCR) is expressed by endothelial cells, myeloid cells, and placental trophoblasts. With phosphatidylcholine (PC) in its antigen-presenting groove, EPCR activates protein C and can act as the co-receptor for TF-FVIIa-FXa-PAR2 signaling. However, when EPCR is recycled in patients with APS, the PC is replaced by endosomal lysobiphosphatidic acid (LBPA).<sup>39</sup> This EPCR-LBPA not only triggers APAs that interfere with the protein C anticoagulant pathway, but also sensitizes TLR7/8 to generate type 1 interferon inflammatory cytokines that promote B-cell activation and APA production, tissue inflammation, and platelet activation.<sup>40</sup>

Increases in endothelial microparticles are observed in APA plasma<sup>41</sup> and APA sera deposit more immunoglobulin on cultured endothelial cells than control sera. The APAs impair the hydrolysis of arachidonic acid from membrane phospholipids by inhibiting thrombin-stimulated phospholipase A<sub>2</sub> activity, thereby reducing the production and release of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation.<sup>42,43</sup> The expression of VWF is stimulated in patients with LAC,<sup>44</sup> and although  $\beta_2$ -GPI binding interferes with VWF-dependent platelet adhesion and aggregation, neutralization of  $\beta_2$ -GPI by anti- $\beta_2$ -GPI antibodies raises VWF levels 1.5-fold.<sup>45</sup>

A cellular receptor for dimeric  $\beta_2$ -GPI is apolipoprotein E2 (apoER2).<sup>46</sup> When complexes of APA and  $\beta_2$ -GPI are bound to apoER2 on the endothelial cell membrane, endothelial nitric oxide synthase (eNOS) is inhibited and endothelial cell-leukocyte adhesion is enhanced.<sup>47</sup> Dephosphorylation of eNOS is mediated by the antibody-induced activation of protein phosphorylase 2A.<sup>48</sup> Impairment of eNOS likely accounts for the decreased nitric oxide metabolites observed in patients with APS.<sup>49</sup> The net effect of APA is to enhance platelet adhesion and diminish the clot-inhibitory properties of the endothelium.

#### Platelets

Thrombocytopenia is occasionally present in APS patients,<sup>50</sup> and is invariably present in those with the catastrophic form of the syndrome.<sup>51</sup> It is accompanied by APAs that bind to platelet antigens and enhance platelet activation and aggregation induced by adenosine diphosphate.<sup>52</sup> Experimental studies show that LAC induces thromboxane A<sub>2</sub> formation, increases urinary excretion of thromboxane B<sub>2</sub> (TXB<sub>2</sub>), activates the endothelium, and binds to platelet thrombi.<sup>53,54</sup> Under flow conditions, APAs augment platelet deposition on the endothelium and the formation of large platelet

**Table 2** Antiphospholipid antibody targets and mechanisms

Target tissue or protein	PL intermediary	Binding site	Pathophysiology
Endothelium	$\beta_2$ -GPI	apoER2', EPCR	Inhibit eNOS, prostacyclin, protein C activation; stimulate VWF
Platelets	$\beta_2$ -GPI, cardiolipin	apoER2', GP1b $\alpha$ , PF4	Induce TxA <sub>2</sub> , microparticles, adhesion, aggregation; upregulate PDI enzymes
Paraoxonase	$\beta_2$ -GPI, cardiolipin	Not established	Increased oxidized LDL, atheromatous disease
Mitochondrial membrane synthase	Oxidized cardiolipin	Not established	Increased type I interferon, accelerated atherosclerosis
Mammalian target of rapamycin	PI-3-kinase	Not established	Endothelial cell proliferation, vascular occlusion; enhanced phosphorylation of AKT kinase
Trophoblasts	Lysobiphosphatidic acid (LBPA)	EPCR; NOD2; mitochondria; complement activation	Stimulate TxA <sub>2</sub> and decrease PGI <sub>2</sub> ; boost secretion of IL-1B and VEGF; block protein C activation, binding of pro-urokinase to its receptors; produce reactive oxygen species; release tissue factor-bearing vesicles from neutrophils
Prothrombin	Phosphatidylserine	Epitopes on prothrombin 1 and fragment 1; less often, epitopes at carboxyl terminus	Enhance Ca <sup>2+</sup> -mediated binding of prothrombin to anionic PL and interfere with antithrombin inhibition of thrombin
Tissue factor	$\beta_2$ -GPI, cardiolipin	Endothelial cells, mononuclear cells	Phosphorylate nonmuscle myosin II regulatory light chain promoting microparticle release, induce TF mRNA, augment factor Xa by inhibiting TFPI
Factor VII/VIIa	–	Not established	Arterial thrombosis
Factor X	–	Not established	Binding of antithrombin to factor Xa impaired
Factor XI	–	Either thioredoxin-1 or protein disulfide isomerase	Increased amount of reduced disulfide bonds in factor XI, accelerating factor XIa generation
Factor XII	PS, cardiolipin	Second growth factor domain, catalytic domain	Impair fibrinolysis, increase arterial and venous thrombosis, obstetrical complications
Kininogen	PE	Not established	Augment thrombin-induced platelet aggregation
Factor XIII	$\beta_2$ -GPI, cardiolipin	Not established	Increased fibrin cross-linking
Protein C	$\beta_2$ -GPI, cardiolipin	Anionic PL	Activated protein C resistance impairing inhibition of factors V and VIII
Protein S	None	EGF domain of protein S	Associated with APCR, thrombosis, and recurrent pregnancy loss
Tissue factor pathway inhibitor	$\beta_2$ -GPI	Anionic PL	Enhanced thrombin generation
Heparin	None	Disaccharide (at antithrombin binding site)	Inhibit heparin-accelerated formation of antithrombin–thrombin complexes
Tissue plasminogen activator, plasminogen activator inhibitor-1, plasmin	Prothrombin, S100A10	Catalytic domain of t-PA	Decreased t-PA activity, increased PAI-1 and TAFI, reduced clot permeability
Complement	$\beta_2$ -GPI, complement factor H	Details of complement activation not established	Deposition of C5b-9, release of proinflammatory and procoagulant cytokines

Abbreviations: apoER2', apolipoprotein E receptor 2';  $\beta_2$ -GPI,  $\beta_2$ -glycoprotein I; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; EPCR, endothelial protein C receptor; GP1b $\alpha$ , glycoprotein I $\alpha$ ; LDL, low density lipoprotein; NOD2, nucleotide-binding oligomerization domain 2; PAI-1, plasminogen activator inhibitor-1; PDI, protein disulfide isomerase; PF4, platelet factor 4; PGI<sub>2</sub>, prostaglandin I<sub>2</sub>; PL, phospholipid; TAFI, thrombin activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; t-PA, tissue plasminogen activator; TxA<sub>2</sub>, thromboxane A<sub>2</sub>; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor.

aggregates<sup>55</sup>; such platelet microparticles are detected in APA patients with thrombosis.<sup>41</sup> In addition, the platelet protein profiles of patients with APA reveal upregulation of protein disulfide isomerase enzymes that favor production of prothrombotic NETS (NETosis) by decreasing levels of platelet SERPINB1.<sup>56</sup>

Ho et al<sup>57</sup> suggest that  $\beta_2$ -GPI attaches to the anionic platelet membrane, assumes the J-shape that enables binding of anti- $\beta_2$ -GPI antibodies, and the complex then interacts with several platelet proteins.  $\beta_2$ -GPI forms complexes with platelet factor 4, and anti- $\beta_2$ -GPI antibodies bind to these complexes and induce platelet p38MAPK phosphorylation and TXB<sub>2</sub> production.<sup>58</sup> Dimers of  $\beta_2$ -GPI mimicking anti- $\beta_2$ -GPI/ $\beta_2$ -GPI complexes bind to the platelet membrane receptor, apoER2', and increase platelet adhesion to collagen and thrombus formation.<sup>59</sup> In addition, anti- $\beta_2$ -GPI/ $\beta_2$ -GPI complexes bind to the platelet GPIIb $\alpha$  receptor and activate platelets.<sup>60</sup> Thus, there are multiple interactions of APA with platelets that are potentially thrombogenic.

### Macrophages

Accelerated (premature) atherosclerosis is another feature of APS.<sup>61</sup> Low density lipoprotein (LDL) family members bind domain V of dimeric  $\beta_2$ -GPI and become targets for APA and anti- $\beta_2$ -GPI.<sup>62</sup> These antibodies decrease the activity of paraoxonase, an enzyme that retards the oxidation of LDL. The decline in paraoxonase correlates with anti- $\beta_2$ -GPI activity<sup>63</sup> and is accompanied by lipid peroxidation, as reflected by increased urinary excretion of isoprostanes.<sup>64</sup> Oxidized LDL uptake by macrophages is enhanced,<sup>65</sup> and the antibodies bind to the oxidized cardiolipin and LDL found in atherosclerotic lesions.<sup>66</sup> Paraoxonase activity is lower in women with APA than in controls ( $p < 0.005$ ), and is inversely associated with carotid intima-media thickness and pulse wave velocity.<sup>67</sup> Immunoglobulin G (IgG) antibodies against oxidized LDL were reported in 47 of 61 (80%) patients with SLE, and roughly correlated with the level of ACA,<sup>68</sup> but are not specifically associated with arterial thromboembolism.<sup>69</sup>

ACAs also target the cardiolipin bound to membrane proteins such as mitochondrial membrane synthase.<sup>70</sup> Monocytes and neutrophils from APS patients have altered mitochondrial membrane potential and evidence of oxidative stress (increased peroxide production, antioxidant enzymatic activity, and decreased intracellular glutathione).<sup>71</sup> Mitochondrial stress releases short DNA fragments into the cytosol, inducing type I interferon production.<sup>72</sup> Notably, the increased expression of platelet type I interferon-regulated proteins is observed in SLE patients with vascular disease.<sup>73</sup> Furthermore, increased interferon- $\alpha$  expression by SLE endothelial progenitor cells and circulating angiogenic cells promotes apoptosis, hampering vessel repair.<sup>74</sup> It seems likely that activation of the type I interferon pathway by antibodies to oxidized cardiolipin contributes to the accelerated atherosclerosis characteristic of patients with the APS.

Indicators of inflammation in APS in addition to interferons are the mammalian target of rapamycin complex (mTORC), IL-4 and IL-6, and activated complement components. APS antibodies are reported to stimulate mTORC through the phos-

phatidylinositol 3-kinase-AKT pathway, enhancing cell proliferation and contributing to renal vascular lesions.<sup>75</sup> Levels of interleukins 4 and 6 are significantly higher in APS patients than in controls with stable coronary disease.<sup>76</sup>

### Trophoblasts

Antibodies to the EPCR have been identified in women with APS, and these antibodies are an independent risk factor for fetal death.<sup>77</sup> In a mouse model, EPCR expression on giant trophoblast cells is essential for fetal viability, presumably because it provides activated protein C to curtail thrombin generation.<sup>78</sup> Fetal loss associated with APA was prevented in mice lacking EPCR signaling, and such mice were also resistant to APA-induced thrombosis.

LAC interferes with the inhibition of factor Va and factor VIIIa by activated protein C, a response that can be corrected by prior incubation of the LAC IgG fractions with phospholipid.<sup>79</sup> Required APA cofactors are either PT in the presence of calcium<sup>80</sup> or  $\beta_2$ -GPI.<sup>81</sup> APA directed against the latter induces activated protein C resistance (APCR) in women with recurrent miscarriages.<sup>82</sup> Autoantibodies that bind to the epidermal growth factor-like domain of protein S have also been identified in patients with recurrent pregnancy loss.<sup>83</sup>

The open form of  $\beta_2$ -GPI is present on decidual endothelium and trophoblasts and can bind anti- $\beta_2$ -GPI antibodies, potentially activating complement.<sup>84</sup> In addition, APA-protein-phospholipid complexes activate complement on neutrophils, stimulating the release of tissue factor-bearing vesicles that contribute to thrombus formation and trophoblast injury.<sup>85</sup> In a mouse model, blocking C5a-C5a receptor interactions on neutrophils prevents fetal injury.<sup>86</sup> Further contributing to placental thrombosis is impairment of fibrinolysis by APAs that inhibit the binding of prourokinase to its trophoblast receptor, and other antibodies that reduce factor XIIIa-dependent profibrinolytic activity.<sup>87,88</sup>

Anti- $\beta_2$ -GPI antibodies target placental mitochondria, induce production of reactive oxygen species, release arachidonic acid and thromboxane A<sub>2</sub>, and bring about cellular damage.<sup>89</sup> They stimulate trophoblast IL-1 $\beta$  and VEGF secretion mediated by nucleotide-binding oligomerization domain-2, potentially accounting for the observed proinflammatory and angiogenic profile in patients with APA.<sup>90</sup>

Recurrent venous and arterial thromboses are also characteristic of obstetrical APS, but whether the same antibodies that promote fetal loss induce vascular thrombi is unclear. Meroni et al<sup>84</sup> suggest that the tissue distribution and expression level of the anti- $\beta_2$ -GPI target antigens could account for the recurrent miscarriages as well as the systemic vascular disease.

In summary, multiple mechanisms contribute to the impaired pregnancy outcomes in women with APS. Antibodies to the EPCR decrease the activation of protein C, resulting in enhanced FVa availability and greater thrombin generation. APAs increase TxA<sub>2</sub> release from trophoblasts and decrease PGI<sub>2</sub> production, reducing placental blood flow. The binding of prourokinase to its trophoblast receptor is

inhibited and antibodies to FXII further impair activation of fibrinolysis. Complement activation by antibodies stimulates the release of tissue factor-bearing vesicles from neutrophils, contributing to thrombus formation. Lastly, anti- $\beta_2$ -GPI antibodies target placental mitochondria and induce production of reactive oxygen species, promoting cellular damage. The consequence is vascular occlusion, tissue infarction, and fetal loss.

## Hemostatic Factors and Complement

### Clotting Factors

Antibodies to PT were reported in 31 of 42 (74%) patients with LAC.<sup>91</sup> The antibodies are heterogeneous; some recognize PT fragment-1 epitopes when the protein is in solution, whereas others require that the molecule be bound to negatively charged phospholipids.<sup>92</sup> They prolong in vitro clotting tests by out-competing factor Xa for phospholipid-binding sites,<sup>30</sup> but in vivo the increased affinity of LAC-PT complexes for phospholipid surfaces augments thrombin production and might contribute to the enhanced risk of thrombosis in patients with SLE.<sup>93,94</sup> Anti-PT antibodies are associated with both arterial and venous thrombosis (odds ratio [OR]: 2.3; 95% confidence interval [CI]: 1.7–3.5).<sup>95</sup> Antibodies that bind to thrombin as well as PT impair the inactivation of thrombin by antithrombin, further increasing the risk of thrombosis.<sup>96</sup> Infrequently, antibodies are directed against epitopes located at the carboxyl terminus of PT; accelerated clearance of the PT antigen-antibody complexes is associated with severe hypoprothrombinemia and bleeding.<sup>97</sup> Interestingly, exposure to bovine thrombin used in conjunction with surgery has produced antibodies to  $\beta_2$ -GPI and cardiolipin as well as to PT and factor V.<sup>98</sup>

ACA induces *tissue factor* messenger RNA (mRNA) in peripheral blood mononuclear and endothelial cells,<sup>99</sup> and soluble tissue factor levels are higher in APS patients than in controls.<sup>100</sup> Anti- $\beta_2$ -GPI antibodies phosphorylate a non-muscle myosin II regulatory light chain, which is required for the release of endothelial cell microparticles and the expression of tissue factor mRNA.<sup>101</sup>

Antibodies to *factor VII/VIIa* are reported in 67% of individuals with APS and are associated with APAs and thrombosis.<sup>102</sup> Sera from 33.9% of APS patients contain antibodies to *factor Xa* that interfere with its inhibition by antithrombin.<sup>103</sup> Patients with APS have upregulated protein disulfide isomerase family members capable of reducing the disulfide bonds of *factor XI*.<sup>56</sup> Reduced factor XI is more readily activated to factor XIa and is increased in APS patients.<sup>104</sup>

Antibodies to *factor XII* are present in 20% of patients with LAC<sup>13</sup> and 40% of patients with SLE, and are associated with arterial and venous thromboses in the latter.<sup>105</sup> Antibody-binding sites are the growth factor and catalytic domains, and PS is generally required for attachment.<sup>106</sup> Other antibodies are reported that prefer phosphatidylethanolamine and recognize *high- and low-molecular-weight kininogens*.<sup>107</sup> These antibodies might be thrombogenic because they impair kininogen-associated inhibition of thrombin-induced platelet aggregation.<sup>108</sup> Lastly, increases in *factor XIIIa* are

strongly associated with APA in patients with thrombosis, and are positively correlated with the levels of plasminogen activator-1 and fibrinogen, as well as with carotid intima-media thickness.<sup>109</sup>

### Anticoagulants

**Protein C:** APAs inhibit the inactivation of factor Va by activated protein C, even in the presence of protein S.<sup>3</sup> Although thrombomodulin levels are increased in APS, presumably because of APA-induced endothelial cell injury, APCR is often encountered.<sup>110</sup> Patients with thrombosis are more likely to have high-avidity anti-protein C antibodies and greater APCR.<sup>111</sup> The binding of aPL-IgG to protein C requires the presence of  $\beta_2$ -GPI and PS.<sup>112</sup> Antibodies against domain I of  $\beta_2$ -GPI are associated with APCR ( $p < 0.0001$ ), and predicted thrombosis in a prospective study of 137 patients with aPL or SLE.<sup>113</sup> As noted previously, binding of LBPA to the EPCR inhibits protein C activation and promotes autoantibody production by activating B-cells.

**Protein S/TFPI:** Protein S levels are significantly lower in individuals with APS than in matched controls,<sup>114</sup> although antibodies to protein S are not detected more frequently (8.1 vs. 4.9%; 95% CI: 0.68–4.43).<sup>115</sup> When autoantibodies to protein S are present, they are associated with APCR (OR: 57.8; 95% CI: 8.53–391) and are a risk factor for deep vein thrombosis (OR: 5.88; 95% CI: 1.96–17.7).<sup>116</sup>

Protein S, in addition to serving as a co-factor for protein C, is also antithrombotic because it enhances the formation of TFPI complexes with factor Xa.<sup>117</sup> However, 18.5% of patients with definite APS were found to have high-titer anti-TFPI activity and their IgG impaired the inhibitory effect of TFPI on factor Xa.<sup>9</sup> Furthermore, the TFPI activity of normal plasma is inhibited by the IgG fractions of 47.5% of patients with SLE.<sup>118</sup> A heightened risk of thrombosis might be anticipated in individuals with a combination of decreased protein S and antibodies to TFPI.

**Heparin:** A specific pentasaccharide sequence in heparin binds antithrombin, producing a conformational change that greatly augments thrombin inhibition. Some patients with APS have antibodies that bind to a disaccharide within the pentasaccharide sequence and inhibit the heparin-accelerated formation of antithrombin-thrombin complexes.<sup>119</sup>

### Fibrinolytic Factors

Fibrinolysis, the dissolution of thrombi, occurs when plasmin is produced by a complex of t-PA, plasminogen, annexin A<sub>2</sub>, and S100A10 assembled on the surface of endothelial cells,<sup>120</sup> and is mainly regulated by plasminogen activator inhibitor-1 (PAI-1), thrombin-activatable fibrinolytic inhibitor (TAFI), and antiplasmin. Several of these components are impacted by APA. Antibodies directed against the catalytic domain of t-PA have been detected in APS patients, producing higher antigen and lower activity levels.<sup>121</sup> Plasma levels of PAI-1 and TAFI are increased and associated with arterial thrombosis in APS patients with elevated lipoprotein(a) or TAFI activation.<sup>122,123</sup> Antibodies to S100A10 are observed in 11.9% of APS patients but only in 1.7% of healthy persons ( $p = 0.01$ ),<sup>124</sup> and might interfere with the assembly of the

plasminogen activation complex on the cell surface. In addition, antiplasmin antibodies are reported in 28% of APS patients.<sup>125</sup> Lastly, fibrin clot permeability and susceptibility to lysis are reduced and clot lysis times are prolonged in patients with high levels of anti-PT antibodies, and are predictive of thromboembolism.<sup>126</sup>

### Complement

Complement activation, recognized by bioassay and detection of C5b-9 deposition on cell surfaces, is present in about a third of APS samples, occurs mainly in conjunction with triple positivity (positive tests for LAC, ACA, and anti- $\beta_2$ -GPI), and is associated with thrombotic events.<sup>127</sup> Increases in C5a are accompanied by decreases in clot permeability and fibrinolysis,<sup>128</sup> and complement components stimulate monocytes and endothelial cells to release pro-inflammatory and procoagulant cytokines.<sup>129</sup> Components are activated by APA-protein-phospholipid complexes, and activated complement components promote the release of cell membrane; these vesicles initiate coagulation by exposing tissue factor and provide a surface for the assembly of the prothrombinase enzyme complex.<sup>130,131</sup> A recent study found evidence of cell surface deposition of complement components 5b-9 in 6 of 7 catastrophic APS patients, most of whom had thromboses and organ infarcts.<sup>127</sup> Furthermore, germline variants of complement regulatory genes were observed in 6 of 10 patients, potentially contributing to uncontrolled complement activation and vascular occlusion in these individuals.

### Limitations

The APL antibodies described in the older studies were often incompletely characterized, affecting the interpretation of the experimental results. Current research has shown that the antibodies in APS patients are heterogeneous, with subpopulations among the major categories (anti- $\beta_2$ -GPI, anti-PS/PT/LAC, ACA) and a large variety of target epitopes. Nevertheless, these papers are included because they helped to define this complex syndrome and laid the groundwork for future investigations.

### Future Directions

The management of APS patients has included long-term anticoagulation, corticosteroids, cytotoxic agents, and immune response modifiers, but none of these modalities have been entirely satisfactory. The vast array of autoantibodies and the many distinctive pathophysiologic processes might require a different approach, perhaps based on reprogramming antibody production. Recent studies of patients with coronavirus infections suggest that direct antibody synthesis occurs in extrafollicular B-cells, bypassing the multiple checkpoints that generally eliminate autoantibodies produced in germinal centers.<sup>132</sup> If direct antibody synthesis is documented in APS, selective targeting of aberrant B-cells could reduce the titer of the autoantibodies.

Autoantibodies might be triggered in some patients with APS if disruption of the nuclear or mitochondrial<sup>72</sup> envelope releases DNA into the cytosol. Cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) forms complexes with cytoplasmic DNA that elicit an immune response. The barrier-to-autointegration factor 1 out-competes cGAS for binding to DNA and appears to protect against aberrant immune responses.<sup>133</sup> Whether this mechanism could be adapted to limit autoantibody production in APS needs to be investigated.

A vaccine approach should also be considered. Krienke et al<sup>134</sup> describe the preparation of a nanoparticle-formulated mRNA coding for disease-related autoantigens that was targeted to lymphoid dendritic cells in a mouse model of experimental autoimmune encephalomyelitis (EAE). This mRNA vaccine promoted antigen presentation on splenic CD11c cells in the absence of co-stimulatory signals. It led to decreased effector T-cells, expanded the development of T-regulatory cells that suppressed autoreactivity, and reduced the severity of established EAE. Identifying specific autoantigens in patients with APS and preparing mRNA vaccines against these autoantigens is another strategy that might control this destructive disorder.

### Conflict of Interest

None declared.

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