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, β_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 β_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[1] 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-dylserine/prothrombin (anti-PS/PT) antibodies,[2] and a variety of other autoantibodies were identified that are directed against complexes of phospholipid, β_2-glycoprotein I (β_2-GPI), and other phospholipid-binding proteins.[3,4] 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

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[5] reported that 34% were drug-associated (chlorpromazine, quinidine, phenytoin, procainamide), 13% autoimmune, 10% infections, and 43% miscellaneous. Greaves[6] 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[7] 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.[8,9] 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[10] reported cerebral infarcts and antibodies to anti-β_2-GPI and cardiolipin in three patients, and Harzallah et al[11] 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 50 IU/dL in 7% of 216 patients.[12] Decreased factor XII has been observed previously in 20.9% of patients with LAC.[13] 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%, β_2-GPI in 4.1%, and PS/PT in 12%.[14] 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 complications 9.5%,[15] 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.[16,17] Autopsy data reveal megakaryocytes and platelet-fibrin thrombi in the lungs, heart, and kidneys.[18] However, major thrombotic events are not associated with the APA, and the β_2-GPI epitopes targeted by the antibodies differ from those observed in patients with APS.[19] 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.[20] 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 Ca^{2+}-mediated binding to negatively charged phospholipids ($\blacktriangleright$**Table 1**). Annexin II mediates the assembly of plasminogen and tissue plasminogen activator (t-PA) on cell membranes, enhancing tissue-based fibrinolysis.[21] β_2-GPI binds to annexin II on the endothelial cell surface. In people susceptible to the APS, the β_2-GPI–annexin II complex might stimulate anti-β_2-GPI antibody formation. Activation of their endothelial cells occurs when the anti-β_2-GPI antibodies cross-link β_2-GPI bound to annexin II.[22] These antibodies are thrombogenic because they not only inhibit surface plasmin expression but also stimulate the release of tissue factor.[23]

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).[24] This annexin shield is disrupted by APA bound to epitope G40-R43 on domain I of β_2-GPI.[25] Circulating apoptotic endothelial cells bearing annexin V are increased in young women with SLE, and are associated with elevated levels of tissue factor.[26] 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.[27]

Table 1 Major phospholipid (PL)-binding proteins

Protein	Size	Location	Description
Annexins	36 kd, 70 aa, repeats in α − helix	II: cell granules, membranes, rafts V: placenta	Ca^{2+}-dependent PL binding; II binds S100A10, t-PA
β_2-Glycoprotein I	48 kd, 326 aa	Plasma: 200 µg/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.

β₂-Glycoprotein I

β₂-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.[28] Immunogenicity is attributed to exposure of these epitopes as well as oxidation of the terminal sulfhydryl groups of β₂-GPI.[4] The developing antibodies target various domains of β₂-GPI; those directed against a domain I epitope comprising Lys39 and Arg43 have LAC activity.[29] This is because these β₂-GPI–antibody complexes can directly interact with factor V, attenuating its activation by factor Xa.[30]

β₂-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.[31,32] The antithrombotic functions of β₂-GPI are impaired by the development of antibodies to the protein. Furthermore, β₂-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.[33] ACA, measured by immunoassay, is closely correlated with LAC as assessed by prolongation of the activated partial thromboplastin time ($r = 0.7$).[34]

Vimentin/Cardiolipin Complexes

Patients with clinical features suggesting the presence of APA but with negative tests for LAC, ACA, and anti-β₂-GPI might have antibodies to a complex of vimentin and cardiolipin.[35] 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-κB). 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-κB.[36,37]

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.[38] 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).[39] 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.[40]

Increases in endothelial microparticles are observed in APA plasma[41] 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 A₂ activity, thereby reducing the production and release of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation.[42,43] The expression of VWF is stimulated in patients with LAC,[44] and although β₂-GPI binding interferes with VWF-dependent platelet adhesion and aggregation, neutralization of β₂-GPI by anti-β₂-GPI antibodies raises VWF levels 1.5-fold.[45]

A cellular receptor for dimeric β₂-GPI is apolipoprotein E2 (apoER2).[46] When complexes of APA and β₂-GPI are bound to apoER2 on the endothelial cell membrane, endothelial nitric oxide synthase (eNOS) is inhibited and endothelial cell–leukocyte adhesion is enhanced.[47] Dephosphorylation of eNOS is mediated by the antibody-induced activation of protein phosphorylase 2A.[48] Impairment of eNOS likely accounts for the decreased nitric oxide metabolites observed in patients with APS.[49] 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,[50] and is invariably present in those with the catastrophic form of the syndrome.[51] It is accompanied by APAs that bind to platelet antigens and enhance platelet activation and aggregation induced by adenosine diphosphate.[52] Experimental studies show that LAC induces thromboxane A₂ formation, increases urinary excretion of thromboxane B₂ (TXB₂), activates the endothelium, and binds to platelet thrombi.[53,54] 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	β_2-GPI	apoER2′, EPCR	Inhibit eNOS, prostacyclin, protein C activation; stimulate VWF
Platelets	β_2-GPI, cardiolipin	apoER2′, GP1bα, PF4	Induce TxA$_2$, microparticles, adhesion, aggregation; upregulate PDI enzymes
Paraoxonase	β_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$_2$ and decrease PGI$_2$; 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 prethrombin 1 and fragment 1; less often, epitopes at carboxyl terminus	Enhance Ca^{2+}-mediated binding of prothrombin to anionic PL and interfere with antithrombin inhibition of thrombin
Tissue factor	β_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	β_2-GPI, cardiolipin	Not established	Increased fibrin cross-linking
Protein C	β_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	β_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	β_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′; β_2-GPI, β_2-glycoprotein I; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; EPCR, endothelial protein C receptor; GP1bα, glycoprotein Ibα; 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$_2$, prostaglandin I$_2$; PL, phospholipid; TAFI, thrombin activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; t-PA, tissue plasminogen activator; TxA$_2$, thromboxane A$_2$; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor.

aggregates[55]; such platelet microparticles are detected in APA patients with thrombosis.[41] 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.[56]

Ho et al[57] suggest that β_2-GPI attaches to the anionic platelet membrane, assumes the J-shape that enables binding of anti-β_2-GPI antibodies, and the complex then interacts with several platelet proteins. β_2-GPI forms complexes with platelet factor 4, and anti-β_2-GPI antibodies bind to these complexes and induce platelet p38MAPK phosphorylation and TXB$_2$ production.[58] Dimers of β_2-GPI mimicking anti-β_2-GPI/β_2-GPI complexes bind to the platelet membrane receptor, apoER2′, and increase platelet adhesion to collagen and thrombus formation.[59] In addition, anti-β_2-GPI/β_2-GPI complexes bind to the platelet GPIbα receptor and activate platelets.[60] Thus, there are multiple interactions of APA with platelets that are potentially thrombogenic.

Macrophages

Accelerated (premature) atherosclerosis is another feature of APS.[61] Low density lipoprotein (LDL) family members bind domain V of dimeric β_2-GPI and become targets for APA and anti-β_2-GPI.[62] These antibodies decrease the activity of paraoxonase, an enzyme that retards the oxidation of LDL. The decline in paraoxonase correlates with anti-β_2-GPI activity[63] and is accompanied by lipid peroxidation, as reflected by increased urinary excretion of isoprostanes.[64] Oxidized LDL uptake by macrophages is enhanced,[65] and the antibodies bind to the oxidized cardiolipin and LDL found in atherosclerotic lesions.[66] 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.[67] 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,[68] but are not specifically associated with arterial thromboembolism.[69]

ACAs also target the cardiolipin bound to membrane proteins such as mitochondrial membrane synthase.[70] 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).[71] Mitochondrial stress releases short DNA fragments into the cytosol, inducing type I interferon production.[72] Notably, the increased expression of platelet type I interferon-regulated proteins is observed in SLE patients with vascular disease.[73] Furthermore, increased interferon-α expression by SLE endothelial progenitor cells and circulating angiogenic cells promotes apoptosis, hampering vessel repair.[74] 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.[75] Levels of interleukins 4 and 6 are significantly higher in APS patients than in controls with stable coronary disease.[76]

Trophoblasts

Antibodies to the EPCR have been identified in women with APS, and these antibodies are an independent risk factor for fetal death.[77] 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.[78] 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.[79] Required APA cofactors are either PT in the presence of calcium[80] or β_2-GPI.[81] APA directed against the latter induces activated protein C resistance (APCR) in women with recurrent miscarriages.[82] Autoantibodies that bind to the epidermal growth factor-like domain of protein S have also been identified in patients with recurrent pregnancy loss.[83]

The open form of β_2-GPI is present on decidual endothelium and trophoblasts and can bind anti-β_2-GPI antibodies, potentially activating complement.[84] 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.[85] In a mouse model, blocking C5a–C5a receptor interactions on neutrophils prevents fetal injury.[86] 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 XIIa-dependent profibrinolytic activity.[87,88]

Anti-β_2-GPI antibodies target placental mitochondria, induce production of reactive oxygen species, release arachidonic acid and thromboxane A$_2$, and bring about cellular damage.[89] They stimulate trophoblast IL-1β and VEGF secretion mediated by nucleotide-binding oligomerization domain-2, potentially accounting for the observed proinflammatory and angiogenic profile in patients with APA.[90]

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[84] suggest that the tissue distribution and expression level of the anti-β_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$_2$ release from trophoblasts and decrease PGI$_2$ 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-β_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.[91] 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.[92] They prolong in vitro clotting tests by out-competing factor Xa for phospholipid-binding sites,[30] 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.[93,94] Anti-PT antibodies are associated with both arterial and venous thrombosis (odds ratio [OR]: 2.3; 95% confidence interval [CI]: 1.7–3.5).[95] Antibodies that bind to thrombin as well as PT impair the inactivation of thrombin by antithrombin, further increasing the risk of thrombosis.[96] 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.[97] Interestingly, exposure to bovine thrombin used in conjunction with surgery has produced antibodies to β_2-GPI and cardiolipin as well as to PT and factor V.[98]

ACA induces *tissue factor* messenger RNA (mRNA) in peripheral blood mononuclear and endothelial cells,[99] and soluble tissue factor levels are higher in APS patients than in controls.[100] Anti-β_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.[101]

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

Antibodies to *factor XII* are present in 20% of patients with LAC[13] and 40% of patients with SLE, and are associated with arterial and venous thromboses in the latter.[105] Antibody-binding sites are the growth factor and catalytic domains, and PS is generally required for attachment.[106] Other antibodies are reported that prefer phosphatidylethanolamine and recognize *high- and low-molecular-weight kininogens*.[107] These antibodies might be thrombogenic because they impair kininogen-associated inhibition of thrombin-induced platelet aggregation.[108] 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.[109]

Anticoagulants

Protein C: APAs inhibit the inactivation of factor Va by activated protein C, even in the presence of protein S.[3] Although thrombomodulin levels are increased in APS, presumably because of APA-induced endothelial cell injury, APCR is often encountered.[110] Patients with thrombosis are more likely to have high-avidity anti-protein C antibodies and greater APCR.[111] The binding of aPL-IgG to protein C requires the presence of β_2-GPI and PS.[112] Antibodies against domain I of β_2-GPI are associated with APCR ($p < 0.0001$), and predicted thrombosis in a prospective study of 137 patients with aPL or SLE.[113] 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,[114] although antibodies to protein S are not detected more frequently (8.1 vs. 4.9%; 95% CI: 0.68–4.43).[115] 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).[116]

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.[117] 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.[9] Furthermore, the TFPI activity of normal plasma is inhibited by the IgG fractions of 47.5% of patients with SLE.[118] 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.[119]

Fibrinolytic Factors

Fibrinolysis, the dissolution of thrombi, occurs when plasmin is produced by a complex of t-PA, plasminogen, annexin A_2, and S100A10 assembled on the surface of endothelial cells,[120] 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.[121] Plasma levels of PAI-1 and TAFI are increased and associated with arterial thrombosis in APS patients with elevated lipoprotein(a) or TAFI activation.[122,123] Antibodies to S100A10 are observed in 11.9% of APS patients but only in 1.7% of healthy persons ($p = 0.01$),[124] 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.[125] 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.[126]

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-β_2-GPI), and is associated with thrombotic events.[127] Increases in C5a are accompanied by decreases in clot permeability and fibrinolysis,[128] and complement components stimulate monocytes and endothelial cells to release pro-inflammatory and procoagulant cytokines.[129] 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.[130,131] 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.[127] 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-β_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 reprograming 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.[132] 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[72] 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.[133] 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[134] 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.

References

1 Hughes GRV. Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. Br Med J (Clin Res Ed) 1983;287 (6399):1088–1089

2 Tonello M, Bison E, Cattini MG, et al. Anti-phosphatidyl-serine/-prothrombin antibodies (aPS/PT) in isolated lupus anticoagulant (LA): is their presence linked to dual test positivity? Clin Chem Lab Med 2021;59(12):1950–1953

3 Oosting JD, Derksen RHWM, Bobbink IWG, Hackeng TM, Bouma BN, de Groot PG. Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C, or protein S: an explanation for their pathogenic mechanism? Blood 1993;81(10):2618–2625

4 Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. N Engl J Med 2013;368(11):1033–1044

5 Triplett DA, Brandt JT, Musgrave KA, Orr CA. The relationship between lupus anticoagulants and antibodies to phospholipid. JAMA 1988;259(04):550–554

6 Greaves M. Antiphospholipid antibodies and thrombosis. Lancet 1999;353(9161):1348–1353

7 Campbell AL, Pierangeli SS, Wellhausen S, Harris EN. Comparison of the effects of anticardiolipin antibodies from patients with the antiphospholipid syndrome and with syphilis on platelet activation and aggregation. Thromb Haemost 1995;73(03):529–534

8 Forastiero RR, Martinuzzo ME, De Larrañaga G, Broze GJ. Antibodies to tissue factor pathway inhibitor are uncommonly detected in patients with infection-related antiphospholipid antibodies. J Thromb Haemost 2003;1(10):2250–2251

9 Forastiero RR, Martinuzzo ME, Broze GJ Jr. High titers of autoantibodies to tissue factor pathway inhibitor are associated with the antiphospholipid syndrome. J Thromb Haemost 2003;1(04):718–724

10 Zhang Y, Xiao M, Zhang S, et al. Coagulopathy and antiphospholipid antibodies win patients with covid-19. N Engl J Med 2020; 382(17):e38

11 Harzallah I, Debliquis A, Drénou B. Lupus anticoagulant is frequent in patients with Covid-19. J Thromb Haemost 2020; 18(08):2064–2065

12 Bowles L, Platton S, Yartey N, et al. Lupus anticoagulant and abnormal coagulation tests in patients with Covid-19. N Engl J Med 2020;383(03):288–290

13 Jones DW, Gallimore MJ, Harris SL, Winter M. Antibodies to factor XII associated with lupus anticoagulant. Thromb Haemost 1999;81(03):387–390

14 Zuo Y, Estes SK, Ali RA, et al. Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. Sci Transl Med 2020;12(570):eabd3876

15 Al-Samkari H, Karp Leaf RS, Dzik WH, et al. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. Blood 2020;136(04):489–500

16 Klok FA, Kruip MJHA, van der Meer NJM, et al. Incidence of thrombotic complications in critically ill ICU patients with Covid-19. Thromb Res 2020;191:145–147

17 Althaus K, Marini I, Zlamal J, et al. Antibody-induced procoagulant platelets in severe COVID-19 infection. Blood 2021;137(08): 1061–1071

18 Rapkiewicz AV, Mai X, Cardsons SE, et al. Megakaryocytes and platelet-fibrin thrombi characterize multi-organ thrombosis at autopsy in Covid-19: a case series. EClinicalMedicine 2020; 24:100434

19 Borghi MO, Beltagy A, Garrafa E, et al. Anti-phospholipid antibodies in COVID-19 are different from those detectable in the anti-phospholipid syndrome. Front Immunol 2020;11:584241

20 Connell NT, Battinelli EM, Connors JM. Coagulopathy of COVID-19 and antiphospholipid antibodies. J Thromb Haemost 2020. Doi: 10.1111/JTH.14893

21 Brownstein C, Deora AB, Jacovina AT, et al. Annexin II mediates plasminogen-dependent matrix invasion by human monocytes: enhanced expression by macrophages. Blood 2004;103(01): 317–324

22 Zhang J, McCrae KR. Annexin A2 mediates endothelial cell activation by antiphospholipid/anti-β2 glycoprotein I antibodies. Blood 2005;105(05):1964–1969

23 Romay-Penabad Z, Montiel-Manzano MG, Shilagard T, et al. Annexin A2 is involved in antiphospholipid antibody-mediated pathogenic effects in vitro and in vivo. Blood 2009;114(14): 3074–3083

24 Andree HAM, Stuart MC, Hermens WT, et al. Clustering of lipid-bound annexin V may explain its anticoagulant effect. J Biol Chem 1992;267(25):17907–17912

25 de Laat B, Wu X-X, van Lummel M, Derksen RHWM, de Groot PG, Rand JH. Correlation between antiphospholipid antibodies that recognize domain I of β2-glycoprotein I and a reduction in the anticoagulant activity of annexin A5. Blood 2007;109(04): 1490–1494

26 Rajagopalan S, Somers EC, Brook RD, et al. Endothelial cell apoptosis in systemic lupus erythematosus: a common pathway for abnormal vascular function and thrombosis propensity. Blood 2004;103(10):3677–3683

27 Rand JH, Wu X-X, Andree HAM, et al. Pregnancy loss in the antiphospholipid-antibody syndrome–a possible thrombogenic mechanism. N Engl J Med 1997;337(03):154–160

28 Agar C, van Os GMA, Mörgelin M, et al. β2-glycoprotein I can exist in 2 conformations: implications for our understanding of the antiphospholipid syndrome. Blood 2010;116(08):1336–1343

29 de Laat B, Derksen RHWM, Urbanus RT, de Groot PG. IgG antibodies that recognize epitope Gly40-Arg43 in domain I of β 2-glycoprotein I cause LAC, and their presence correlates strongly with thrombosis. Blood 2005;105(04):1540–1545

30 Noordermeer T, Molhoek JE, Schutgens REG, et al. Anti-β2-glycoprotein I and anti-prothrombin antibodies cause lupus anticoagulant through different mechanisms of action. J Thromb Haemost 2021;19(04):1018–1028

31 de Groot PG, Meijers JC. β(2)-Glycoprotein I: evolution, structure and function. J Thromb Haemost 2011;9(07):1275–1284

32 Merrill JT, Zhang HW, Shen C, et al. Enhancement of protein S anticoagulant function by β2-glycoprotein I, a major target antigen of antiphospholipid antibodies: β2-glycoprotein I interferes with binding of protein S to its plasma inhibitor, C4b-binding protein. Thromb Haemost 1999;81(05):748–757

33 Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. Ann Intern Med 1990;112(09):682–698

34 Harris EN, Loizou S, Englert H, et al. Anticardiolipin antibodies and lupus anticoagulant. Lancet 1984;2(8411):1099

35 Ortona E, Capozzi A, Colasanti T, et al. Vimentin/cardiolipin complex as a new antigenic target of the antiphospholipid syndrome. Blood 2010;116(16):2960–2967

36 Vega-Ostertag M, Casper K, Swerlick R, Ferrara D, Harris EN, Pierangeli SS. Involvement of p38 MAPK in the up-regulation of tissue factor on endothelial cells by antiphospholipid antibodies. Arthritis Rheum 2005;52(05):1545–1554

37 Espinola RG, Liu X, Colden-Stanfield M, Hall J, Harris EN, Pierangeli SS. E-Selectin mediates pathogenic effects of antiphospholipid antibodies. J Thromb Haemost 2003;1(04):843–848

38 Comfurius P, Bevers EM, Galli M, Zwaal RFA. Regulation of phospholipid asymmetry and induction of antiphospholipid antibodies. Lupus 1995;4(Suppl 1):S19–S22

39 Müller-Calleja N, Hollerbach A, Royce J, et al. Lipid presentation by the protein C receptor links coagulation with autoimmunity. Science 2021;371(6534):1121–1134

40 Kaplan MJ. Linking clotting and autoimmunity. Science 2021; 371(6534):1100–1101

41 Jy W, Tiede M, Bidot CJ, et al. Platelet activation rather than endothelial injury identifies risk of thrombosis in subjects positive for antiphospholipid antibodies. Thromb Res 2007;121(03): 319–325

42 Carreras LO, Defreyn G, Machin SJ, et al. Arterial thrombosis, intrauterine death and "lupus" antiocoagulant: detection of immunoglobulin interfering with prostacyclin formation. Lancet 1981;1(8214):244–246

43 Schorer AE, Duane PG, Woods VL, Niewoehner DE. Some antiphospholipid antibodies inhibit phospholipase A2 activity. J Lab Clin Med 1992;120(01):67–77

44 McCrae KR, DeMichele A, Samuels P, et al. Detection of endothelial cell-reactive immunoglobulin in patients with anti-phospholipid antibodies. Br J Haematol 1991;79(04):595–605

45 Hulstein JJJ, Lenting PJ, de Laat B, Derksen RHWM, Fijnheer R, de Groot PG. β2-Glycoprotein I inhibits von Willebrand factor dependent platelet adhesion and aggregation. Blood 2007;110 (05):1483–1491

46 Romay-Penabad Z, Aguilar-Valenzuela R, Urbanus RT, et al. Apolipoprotein E receptor 2 is involved in the thrombotic complications in a murine model of the antiphospholipid syndrome. Blood 2011;117(04):1408–1414

47 Ramesh S, Morrell CN, Tarango C, et al. Antiphospholipid antibodies promote leukocyte-endothelial cell adhesion and thrombosis in mice by antagonizing eNOS via β2GPI and apoER2. J Clin Invest 2011;121(01):120–131

48 Sacharidou A, Chambliss KL, Ulrich V, et al. Antiphospholipid antibodies induce thrombosis by PP2A activation via apoER2-Dab2-SHC1 complex formation in endothelium. Blood 2018;131 (19):2097–2110

49 Ames PRJ, Batuca JR, Ciampa A, Iannaccone L, Delgado Alves J. Clinical relevance of nitric oxide metabolites and nitrative stress in thrombotic primary antiphospholipid syndrome. J Rheumatol 2010;37(12):2523–2530

50 Comellas-Kirkerup L, Hernández-Molina G, Cabral AR. Antiphospholipid-associated thrombocytopenia or autoimmune hemolytic anemia in patients with or without definite primary

antiphospholipid syndrome according to the Sapporo revised classification criteria: a 6-year follow-up study. Blood 2010;116 (16):3058–3063

51 Pontara E, Banzato A, Bison E, et al. Thrombocytopenia in high-risk patients with antiphospholipid syndrome. J Thromb Haemost 2018;16(03):529–532

52 Nojima J, Suehisa E, Kuratsune H, et al. Platelet activation induced by combined effects of anticardiolipin and lupus anticoagulant IgG antibodies in patients with systemic lupus erythematosus–possible association with thrombotic and thrombocytopenic complications. Thromb Haemost 1999;81 (03):436–441

53 Lellouche F, Martinuzzo M, Said P, Maclouf J, Carreras LO. Imbalance of thromboxane/prostacyclin biosynthesis in patients with lupus anticoagulant. Blood 1991;78(11):2894–2899

54 Proulle V, Furie RA, Merrill-Skoloff G, Furie BC, Furie B. Platelets are required for enhanced activation of the endothelium and fibrinogen in a mouse thrombosis model of APS. Blood 2014;124 (04):611–622

55 Escolar G, Font J, Reverter JC, et al. Plasma from systemic lupus erythematosus patients with antiphospholipid antibodies promotes platelet aggregation. Studies in a perfusion system. Arterioscler Thromb 1992;12(02):196–200

56 Hell L, Lurger K, Mauracher L-M, et al. Altered platelet proteome in lupus anticoagulant (LA)-positive patients-protein disulfide isomerase and NETosis as new players in LA-related thrombosis. Exp Mol Med 2020;52(01):66–78

57 Ho YC, Ahuja KDK, Körner H, Adams MJ. β₂GPI-anti-β₂GPI antibodies and platelets: key players in the anti-phospholipid syndrome. Antibodies (Basel) 2016;5(02):12

58 Sikara MP, Routsias JG, Samiotaki M, Panayotou G, Moutsopoulos HM, Vlachoyiannopoulos PG. beta2 Glycoprotein I (beta2GPI) binds platelet factor 4 (PF4): implications for the pathogenesis of antiphospholipid syndrome. Blood 2010;115(03):713–723

59 Lutters BC, Derksen RH, Tekelenburg WL, Lenting PJ, Arnout J, de Groot PG. Dimers of beta 2-glycoprotein I increase platelet deposition to collagen via interaction with phospholipids and the apolipoprotein E receptor 2′. J Biol Chem 2003;278(36): 33831–33838

60 Shi T, Giannakopoulos B, Yan X, et al. Anti-β2-glycoprotein I antibodies in complex with β2-glycoprotein I can activate platelets in a dysregulated manner via glycoprotein Ib-IX-V. Arthritis Rheum 2006;54(08):2558–2567

61 Ames PRJ, Antinolfi I, Scenna G, Gaeta G, Margaglione M, Margarita A. Atherosclerosis in thrombotic primary antiphospholipid syndrome. J Thromb Haemost 2009;7(04):537–542

62 Pennings MT, van Lummel M, Derksen RH, et al. Interaction of beta2-glycoprotein I with members of the low density lipoprotein receptor family. J Thromb Haemost 2006;4(08):1680–1690

63 Alves JD, Ames PRJ, Donohue S, et al. Antibodies to high-density lipoprotein and β₂-glycoprotein I are inversely correlated with paraoxonase activity in systemic lupus erythematosus and primary antiphospholipid syndrome. Arth Rheum 2002; 46:2686–2694

64 Iuliano L, Praticò D, Ferro D, et al. Enhanced lipid peroxidation in patients positive for antiphospholipid antibodies. Blood 1997;90 (10):3931–3935

65 Hasunuma Y, Matsuura E, Makita Z, Katahira T, Nishi S, Koike T. Involvement of β 2-glycoprotein I and anticardiolipin antibodies in oxidatively modified low-density lipoprotein uptake by macrophages. Clin Exp Immunol 1997;107(03):569–573

66 An T, Miller YI, Hansen LF, Kesaniemi YA, Witztum JL, Horkko S. A natural antibody to oxidized cardiolipin binds to oxidized low-density lipoprotein, apoptotic cells, and atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2006;26(09):2096–2102

67 Charakida M, Besler C, Batuca JR, et al. Vascular abnormalities, paraoxonase activity, and dysfunctional HDL in primary antiphospholipid syndrome. JAMA 2009;302(11):1210–1217

68 Vaarala O, Alfthan G, Jauhiainen M, Leirisalo-Repo M, Aho K, Palosuo T. Crossreaction between antibodies to oxidised low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. Lancet 1993;341(8850):923–925

69 Pengo V, Bison E, Ruffatti A, Iliceto S. Antibodies to oxidized LDL/ β2-glycoprotein I in antiphospholipid syndrome patients with venous and arterial thromboembolism. Thromb Res 2008;122 (04):556–559

70 Whitelegge J. Structural biology. Up close with membrane lipid-protein complexes. Science 2011;334(6054):320–321

71 Perez-Sanchez C, Ruiz-Limon P, Aguirre MA, et al. Mitochondrial dysfunction in antiphospholipid syndrome: implications in the pathogenesis of the disease and effects of coenzyme Q(10) treatment. Blood 2012;119(24): 5859–5870

72 Kim J, Gupta R, Blanco LP, et al. VDAC oligomers form mitochondrial pores to release mtDNA fragments and promote lupus-like disease. Science 2019;366(6472):1531–1536

73 Lood C, Amisten S, Gullstrand B, et al. Platelet transcriptional profile and protein expression in patients with systemic lupus erythematosus: up-regulation of the type I interferon system is strongly associated with vascular disease. Blood 2010;116(11): 1951–1957

74 Denny MF, Thacker S, Mehta H, et al. Interferon-α promotes abnormal vasculogenesis in lupus: a potential pathway for premature atherosclerosis. Blood 2007;110(08):2907–2915

75 Canaud G, Bienaimé F, Tabarin F, et al. Inhibition of the mTORC pathway in the antiphospholipid syndrome. N Engl J Med 2014; 371(04):303–312

76 Soltesz P, Der H, Veres K, et al. Immunological features of primary anti-phospholipid syndrome in connection with endothelial dysfunction. Rheumatology (Oxford) 2008;47(11):1628–1634

77 Hurtado V, Montes R, Gris J-C, et al. Autoantibodies against EPCR are found in antiphospholipid syndrome and are a risk factor for fetal death. Blood 2004;104(05):1369–1374

78 Li W, Zheng X, Gu J-M, et al. Extraembryonic expression of EPCR is essential for embryonic viability. Blood 2005;106(08): 2716–2722

79 Pötzsch B, Kawamura H, Preissner KT, Schmidt M, Seelig C, Müller-Berghaus G. Acquired protein C dysfunction but not decreased activity of thrombomodulin is a possible marker of thrombophilia in patients with lupus anticoagulant. J Lab Clin Med 1995;125(01):56–65

80 Field SL, Chesterman CN, Hogg PJ. Dependence on prothrombin for inhibition of activated protein C activity by lupus antibodies. Thromb Haemost 2000;84(06):1132–1133

81 Galli M, Ruggeri L, Barbui T. Differential effects of anti-beta2-glycoprotein I and antiprothrombin antibodies on the anticoagulant activity of activated protein C. Blood 1998;91(06): 1999–2004

82 Mercier E, Quere I, Mares P, Gris J-C. Primary recurrent miscarriages: anti-β2-glycoprotein I IgG antibodies induce an acquired activated protein C resistance that can be detected by the modified activated protein C resistance test. Blood 1998;92 (08):2993–2994

83 Sato Y, Sugi T, Sakai R. Antigenic binding sites of anti-protein S autoantibodies in patients with recurrent pregnancy loss. Res Pract Thromb Haemost 2018;2(02):357–365

84 Meroni PL, Borghi MO, Grossi C, Chighizola CB, Durigutto P, Tedesco F. Obstetric and vascular antiphospholipid syndrome: same antibodies but different diseases? Nat Rev Rheumatol 2018;14(07):433–440

85 Redecha P, Tilley R, Tencati M, et al. Tissue factor: a link between C5a and neutrophil activation in antiphospholipid antibody induced fetal injury. Blood 2007;110(07):2423–2431

86 Girardi G, Berman J, Redecha P, et al. Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. J Clin Invest 2003;112(11):1644–1654

87 Carmona F, Lázaro I, Reverter JC, et al. Impaired factor XIIa-dependent activation of fibrinolysis in treated antiphospholipid syndrome gestations developing late-pregnancy complications. Am J Obstet Gynecol 2006;194(02):457–465

88 McCrae KR, DeMichele AM, Pandhi P, et al. Detection of antitrophoblast antibodies in the sera of patients with anticardiolipin antibodies and fetal loss. Blood 1993;82 (09):2730–2741

89 Zussman R, Xu LY, Damani T, et al. Antiphospholipid antibodies can specifically target placental mitochondria and induce ROS production. J Autoimmun 2020;111:102437

90 Mulla MJ, Pasternak MC, Salmon JE, Chamley LW, Abrahams VM. Role of NOD2 in antiphospholipid antibody-induced and bacterial MDP amplification of trophoblast inflammation. J Autoimmun 2019;98:103–112

91 Fleck RA, Rapaport SI, Rao LVH. Anti-prothrombin antibodies and the lupus anticoagulant. Blood 1988;72(02):512–519

92 Chinnaraj M, Planer W, Pengo V, Pozzi N. Discovery and characterization of 2 novel subpopulations of aPS/PT antibodies in patients at high risk of thrombosis. Blood Adv 2019;3(11): 1738–1749

93 Field SL, Hogg PJ, Daly EB, et al. Lupus anticoagulants form immune complexes with prothrombin and phospholipid that can augment thrombin production in flow. Blood 1999;94(10): 3421–3431

94 Bizzaro N, Ghirardello A, Zampieri S, et al. Anti-prothrombin antibodies predict thrombosis in patients with systemic lupus erythematosus: a 15-year longitudinal study. J Thromb Haemost 2007;5(06):1158–1164

95 Sciascia S, Sanna G, Murru V, Roccatello D, Khamashta MA, Bertolaccini ML. Anti-prothrombin (aPT) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies and the risk of thrombosis in the antiphospholipid syndrome. A systematic review. Thromb Haemost 2014;111(02):354–364

96 Hwang K-K, Grossman JM, Visvanathan S, et al. Identification of anti-thrombin antibodies in the antiphospholipid syndrome that interfere with the inactivation of thrombin by antithrombin. J Immunol 2001;167(12):7192–7198

97 Bajaj SP, Rapaport SI, Fierer DS, Herbst KD, Schwartz DB. A mechanism for the hypoprothrombinemia of the acquired hypoprothrombinemia-lupus anticoagulant syndrome. Blood 1983; 61(04):684–692

98 Su Z, Izumi T, Thames EH, Lawson JH, Ortel TL. Antiphospholipid antibodies after surgical exposure to topical bovine thrombin. J Lab Clin Med 2002;139(06):349–356

99 Amengual O, Atsumi T, Khamashta MA, Hughes GRV. The role of the tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid syndrome. Thromb Haemost 1998;79 (02):276–281

100 Williams FMK, Parmar K, Hughes GRV, Hunt BJ. Systemic endothelial cell markers in primary antiphospholipid syndrome. Thromb Haemost 2000;84(05):742–746

101 Betapudi V, Lominadze G, Hsi L, Willard B, Wu M, McCrae KR. Anti-β2GPI antibodies stimulate endothelial cell microparticle release via a nonmuscle myosin II motor protein-dependent pathway. Blood 2013;122(23):3808–3817

102 Bidot CJ, Jy W, Horstman LL, et al. Factor VII/VIIa: a new antigen in the anti-phospholipid antibody syndrome. Br J Haematol 2003;120(04):618–626

103 Artim-Esen B, Pericleous C, Mackie I, et al. Anti-factor Xa antibodies in patients with antiphospholipid syndrome and their effects upon coagulation assays. Arthritis Res Ther 2015;17:47

104 Giannakopoulos B, Gao L, Qi M, et al. Factor XI is a substrate for oxidoreductases: enhanced activation of reduced FXI and its role in antiphospholipid syndrome thrombosis. J Autoimmun 2012; 39(03):121–129

105 Bertolaccini ML, Mepani K, Sanna G, Hughes GRV, Khamashta MA. Factor XII autoantibodies as a novel marker for thrombosis

and adverse obstetric history in patients with systemic lupus erythematosus. Ann Rheum Dis 2007;66(04):533–536

106 Harris SL, Jones DW, Gallimore MJ, Nicholls PJ, Winter M. The antigenic binding site(s) of antibodies to factor XII associated with the antiphospholipid syndrome. J Thromb Haemost 2005;3 (05):969–975

107 Sugi T, McIntyre JA. Autoantibodies to phosphatidylethanolamine (PE) recognize a kininogen-PE complex. Blood 1995;86 (08):3083–3089

108 Sugi T, McIntyre JA. Autoantibodies to kininogen-phosphatidylethanolamine complexes augment thrombin-induced platelet aggregation. Thromb Res 1996;84(02):97–109

109 Ames PRJ, Iannaccone L, Alves JD, Margarita A, Lopez LR, Brancaccio V. Factor XIII in primary antiphospholipid syndrome. J Rheumatol 2005;32(06):1058–1062

110 Karmochkine M, Boffa MC, Piette JC, et al. Increase in plasma thrombomodulin in lupus erythematosus with antiphospholipid antibodies. Blood 1992;79(03):837–838

111 Arachchillage DRJ, Efthymiou M, Mackie IJ, Lawrie AS, Machin SJ, Cohen H. Anti-protein C antibodies are associated with resistance to endogenous protein C activation and a severe thrombotic phenotype in antiphospholipid syndrome. J Thromb Haemost 2014;12(11):1801–1809

112 Atsumi T, Khamashta MA, Amengual O, et al. Binding of anti-cardiolipin antibodies to protein C via β2-glycoprotein I (β2-GPI): a possible mechanism in the inhibitory effect of antiphospholipid antibodies on the protein C system. Clin Exp Immunol 1998;112(02):325–333

113 Zuily S, de Laat B, Guillemin F, et al. Anti-domain I β2-glycoprotein I antibodies and activated protein C resistance predict thrombosis in antiphospholipid syndrome: TAC(1)T study. J Appl Lab Med 2020;5(06):1242–1252

114 Crowther MA, Johnston M, Weitz J, Ginsberg JS. Free protein S deficiency may be found in patients with antiphospholipid antibodies who do not have systemic lupus erythematosus. Thromb Haemost 1996;76(05):689–691

115 Rossetto V, Spiezia L, Franz F, et al. The role of antiphospholipid antibodies toward the protein C/protein S system in venous thromboembolic disease. Am J Hematol 2009;84(09):594–596

116 Nojima J, Kuratsune H, Suehisa E, et al. Acquired activated protein C resistance associated with anti-protein S antibody as a strong risk factor for DVT in non-SLE patients. Thromb Haemost 2002;88(05):716–722

117 Rosing J, Maurissen LF, Tchaikovski SN, Tans G, Hackeng TM. Protein S is a cofactor for tissue factor pathway inhibitor. Thromb Res 2008;122(Suppl 1):S60–S63

118 Adams MJ, Palatinus AA, Harvey AM, Khalafallah AA. Impaired control of the tissue factor pathway of blood coagulation in systemic lupus erythematosus. Lupus 2011;20(14):1474–1483

119 Shibata S, Harpel PC, Gharavi A, Rand J, Fillit H. Autoantibodies to heparin from patients with antiphospholipid antibody syndrome inhibit formation of antithrombin III-thrombin complexes. Blood 1994;83(09):2532–2540

120 Hajjar KA. The biology of annexin A2: from vascular fibrinolysis to innate immunity. Trans Am Clin Climatol Assoc 2015; 126:144–155

121 Cugno M, Cabibbe M, Galli M, et al. Antibodies to tissue-type plasminogen activator (tPA) in patients with antiphospholipid syndrome: evidence of interaction between the antibodies and the catalytic domain of tPA in 2 patients. Blood 2004;103(06): 2121–2126

122 Singh NK, Gupta A, Behera DR, Dash D. Elevated plasminogen activator inhibitor type-1 (PAI-1) as contributing factor in pathogenesis of hypercoagulable state in antiphospholipid syndrome. Rheumatol Int 2013;33(09):2331–2336

123 Yamazaki M, Asakura H, Jokaji H, et al. Plasma levels of lipoprotein(a) are elevated in patients with the antiphospholipid antibody syndrome. Thromb Haemost 1994;71(04):424–427

124 Salle V, Sagnier A, Diouf M, et al. Prevalence of anti-S100A10 antibodies in antiphospholipid syndrome patients. Thromb Res 2019;179:15–19

125 Yang CD, Hwang KK, Yan W, et al. Identification of anti-plasmin antibodies in the antiphospholipid syndrome that inhibit degradation of fibrin. J Immunol 2004;172(09):5765–5773

126 Ząbczyk M, Celińska-Löwenhoff M, Plens K, Iwaniec T, Musiał J, Undas A. Antiphosphatidylserine/prothrombin complex antibodies as a determinant of prothrombotic plasma fibrin clot properties in patients with antiphospholipid syndrome. J Thromb Haemost 2019;17(10):1746–1755

127 Chaturvedi S, Braunstein EM, Yuan X, et al. Complement activity and complement regulatory gene mutations are associated with thrombosis in APS and CAPS. Blood 2020;135(04):239–251

128 Grosso G, Vikerfors A, Woodhams B, et al. Thrombin activatable fibrinolysis inhibitor (TAFI) - A possible link between coagulation and complement activation in the antiphospholipid syndrome (APS). Thromb Res 2017;158:168–173

129 Chaturvedi S, Braunstein EM, Brodsky RA. Antiphospholipid syndrome: complement activation, complement gene mutations, treatment implications. J Thromb Haemost 2021;19(03):607–616

130 Hamilton KK, Hattori R, Esmon CT, Sims PJ. Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. J Biol Chem 1990;265(07):3809–3814

131 Ikeda K, Nagasawa K, Horiuchi T, Tsuru T, Nishizaka H, Niho Y. C5a induces tissue factor activity on endothelial cells. Thromb Haemost 1997;77(02):394–398

132 Woodruff MC, Ramonell RP, Nguyen DC, et al. Extrafollicular B cell responses correlate with neutralizing antibodies and morbidity in COVID-19. Nat Immunol 2020;21(12):1506–1516

133 Guey B, Wischnewski M, Decout A, et al. BAF restricts cGAS on nuclear DNA to prevent innate immune activation. Science 2020;369(6505):823–828

134 Krienke C, Kolb L, Diken E, et al. A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis. Science 2021;371(6525):145–153