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REVIEW

Mechanisms of immunothrombosis and vasculopathy in antiphospholipid syndrome

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Abstract

Antiphospholipid syndrome (APS) is an autoimmune thrombophilia propelled by circulating antiphospholipid antibodies that herald vascular thrombosis and obstetrical complications. Antiphospholipid antibodies recognize phospholipids and phospholipid-binding proteins and are not only markers of disease but also key drivers of APS pathophysiology. Thrombotic events in APS can be attributed to various conspirators including activated endothelial cells, platelets, and myeloid-lineage cells, as well as derangements in coagulation and fibrinolytic systems. Furthermore, recent work has especially highlighted the role of neutrophil extracellular traps (NETs) and the complement system in APS thrombosis. Beyond acute thrombosis, patients with APS can also develop an occlusive vasculopathy, a long-term consequence of APS characterized by cell proliferation and infiltration that progressively expands the intima and leads to organ damage. This review will highlight known pathogenic factors in APS and will also briefly discuss similarities between APS and the thrombophilic coagulopathy of COVID-19.

Keywords Antiphospholipid syndrome · Thrombosis · Vasculopathy · Neutrophil extracellular traps

Introduction

Antiphospholipid syndrome (**APS**) is a thrombo-inflammatory disease that complicates up to one-third of cases of systemic lupus erythematosus (referred to as "lupus" going forward) where it portends the acquisition of more organ damage over time [1–6]. Meanwhile, the standalone form of APS (primary APS) is even more common, affecting at least 1 in 2000 Americans [7]. APS is propelled by circulating antiphospholipid antibodies (**aPL**) that cause vascular thrombosis and obstetrical complications [8]. Thrombosis in APS may affect vascular beds of all sizes including arterial, venous, and microvascular circuits. Lower extremity deep veins and cerebral arteries are the most frequent sites of venous and arterial thrombosis, respectively [9]. Thrombi may also form in sites uncommonly seen in the general population including arteries that supply the viscera and venous sinuses surrounding the brain. Patients with APS are additionally at risk for microvascular thrombosis in the skin, eyes, heart, lungs, kidneys, and other organs. A minority of patients develop catastrophic APS (CAPS), characterized by microvascular thrombosis in at least three organs, typically all emerging within 1 week [10, 11]. Beyond thrombosis and pregnancy loss, APS is also associated with a variety of extra-criteria manifestations, including livedo reticularis and racemosa, neurologic pathology (cognitive dysfunction, choreiform movements, seizures), valvular heart disease, occlusive vasculopathy, pulmonary hypertension, nephropathy, and thrombocytopenia, among others [12, 13].

In addition to a history of at least one morbid thrombotic or obstetric event, APS classification criteria (Table 1) seek the stable presence of anticardiolipin or anti-beta-2 glycoprotein I (**β_2GPI**) antibodies [8]. Furthermore, the "lupus anticoagulant" test—a functional assay screening for aPL based on prolongation of clotting times—is part of the

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Table 1 Classification criteria for antiphospholipid syndrome [8]

Clinical criteria	Vascular thrombosis	≥ 1 clinical episode of arterial, venous, or small-vessel thrombosis
	Pregnancy morbidity	**a)** ≥ 1 unexplained death of a morphologically normal fetus at ≥ 10 weeks of gestation **b)** ≥ 1 premature delivery of a morphologically normal fetus at < 34 weeks' gestation because of: *i)* Severe preeclampsia or eclampsia defined according to standard definition *ii)* Recognized features of placental insufficiency **c)** ≥ 3 unexplained consecutive miscarriages at < 10-week gestation, with maternal and paternal factors (anatomic, hormonal, or chromosomal abnormalities) excluded
Laboratory criteria	The presence of antiphospholipid antibodies on ≥ 2 occasions ≥ 12 weeks apart **a)** Presence of lupus anticoagulant in plasma **b)** Medium- to high-titer anticardiolipin antibodies of IgG or IgM isotypes **c)** Medium- to high-titer anti-beta-2 glycoprotein I (anti-β_2GPI) antibodies of IgG or IgM isotypes	

classification criteria where it detects a variety of species of aPL including anti-phosphatidylserine/prothrombin antibodies [14]. Modern anticardiolipin assays are designed to recognize anti-β_2GPI antibodies, as β_2GPI protein present in the sample diluent provides a bridge between antibody and cardiolipin [15–17]. Furthermore, some anti-β_2GPI antibodies clearly have lupus anticoagulant activity [18–20]. It should therefore be recognized that a single antibody can potentially turn all three criteria lab tests positive, and the information provided by these different assays is therefore not as granular as one may initially assume.

APS is present if at least one of the clinical criteria and one of the laboratory criteria are met.

It is now recognized that the term "antiphospholipid" is something of a misnomer since the best characterized aPL do not recognize isolated anionic phospholipids such as cardiolipin and phosphatidylserine as originally surmised, but rather specific phospholipid-binding proteins, with aPL targeting the abundant plasma protein β_2GPI particularly pathogenic [21–23]; it is also conceivable that these antibodies detect heterotypic complexes of phospholipids and phospholipid-binding proteins. Intriguingly, the isotype of aPL immunoglobulin abnormality may vary by patient subset. For example, an early study observed that IgA was the most prevalent isotype among Black patients with SLE [24], although the potential pathogenic role of IgA and implications for APS remain to be firmly established.

Life-long anticoagulation is so far the only treatment that has been proven to reduce the vascular complications of APS. However, while anticoagulation regimens are relatively effective in restraining large-vessel events such as deep vein thrombosis and thromboembolic stroke, they do not combat many extra-criteria manifestations such as livedoid vasculopathy, seizures, cognitive decline, alveolar hemorrhage, and thrombocytopenia. Furthermore, anticoagulants do not mitigate the chronic occlusive vasculopathy and progressive organ deterioration that afflict many patients over time. This unmet need is emphasized by an international cohort of more than 800 aPL-positive patients in which 56% of patients had at least one non-thrombotic/non-obstetric manifestation of APS [25]. Notably, more than 25% of these patients were identified as having either white matter brain lesions or premature cognitive dysfunction, and 20% were found to have microvascular disease involving either the kidney or skin. Strategies to combat the long-term, anticoagulant-resistant manifestations of APS are unknown and will likely require new immunomodulatory approaches.

The development of a consensus, unified explanation of APS pathophysiology has unfortunately been hindered by the heterogeneity of aPL profiles (only a fraction of which is likely revealed by standard clinical laboratory testing) and diversity of potential aPL effector functions. As will be discussed below, numerous and wide-ranging "bad actors" have been implicated to date, including blood and immune cells, complement proteins, and coagulation/fibrinolytic systems.

Thrombosis

Notably, aPL are not only markers of disease but also key drivers of APS pathogenesis. Indeed, many manifestations of APS can be reproduced experimentally via transfer of patient serum or immunoglobulins into animals [26–28]. Although the infusion of pathogenic aPL does not cause spontaneous thrombosis in animals, the introduction of some type of disruption to the vasculature (such as injury to the vessel wall; alteration of blood flow; or infusion of lipopolysaccharide, histones, or other immune stimulants) unmasks the exaggerated thromboinflammatory state. The "two-hit" concept of APS (in animal models as well as in patients) posits that aPL provide the first hit, creating a generalized procoagulant state. Subsequently, a second hit (sometimes cryptic) such as a vascular injury or inflammatory stimulus then tips circulating blood toward coagulation. Although this triggering

stimulus is not obvious in many cases of thrombotic APS, a precipitating factor such as surgery, infection, pregnancy, or anticoagulation withdrawal has been identified in 50 to 80% of CAPS episodes [29].

Attention should also be paid to additional risk factors that further increase thrombotic risk in aPL-positive patients [30, 31]. Some potential factors include a concomitant diagnosis of lupus, pregnancy, receipt of estrogen-containing contraceptives, immobilization after surgery, active cancer, heritable thrombophilias, and traditional cardiovascular risk factors such as smoking, hypertension, hypercholesterolemia, and obesity. As an example, one large population-based case–control study found that the odds ratio of ischemic stroke in lupus anticoagulant-positive females was 43.1 (95% confidence interval 12.2 to 152.0), further increasing to 87 (95% confidence interval 14.5 to 523.0) in individuals who smoked and to a remarkable 201 (95% confidence interval 1.9 to 242) in individuals using estrogen-containing oral contraceptives [32].

The earliest identified prothrombotic effects of aPL were via interference with natural anticoagulant systems regulating coagulation and fibrinolysis. However, subsequent studies eventually revealed that a key role of aPL (arguably *the* key role) is to induce activation of various blood and immune cells, as well as the complement system, with procoagulant and proinflammatory consequences. Major pathogenic mechanisms are summarized in Table 2. The relative importance of these factors to a particular thrombotic event is likely dependent on the vascular bed being considered, a concept that will benefit from further mechanistic research.

Endothelial cells. Given its constant confrontation with whole blood, the endothelium necessarily has properties that counter thrombosis and inflammation [62]. For example, heparanoid proteoglycans, prostacyclins, ectonucleotidases such as CD39 and CD73, protein C receptor, and tissue factor pathway inhibitor all help promote an antithrombotic surface [63]. The endothelium is also a barrier that selectively permits molecular and cellular transit from the blood compartment into tissue. When activated, the normally quiescent endothelium sheds its antithrombotic profile and acquires a phenotype that promotes an inflammatory response. Leukocyte-endothelial interactions and extravasation are orchestrated by selectins and cell adhesion molecules that facilitate rolling at the endothelial surface, followed by stronger integrin-mediated interactions that promote adhesion and eventual exodus of leukocytes from vessels [64]. Translational studies have detected endothelium-derived microparticles in the circulation of APS patients as a surrogate for endothelial activation, suggesting the vessel wall may be primed for leukocyte interactions [35, 36].

In vitro, aPL activate healthy cultured endothelial cells to express adhesion molecules and tissue factor [33, 34]. Mechanistically, aPL co-opt pathways normally associated with non-autoimmune inflammatory stimuli. aPL engage apolipoprotein E receptor 2 and possibly other surface receptors on endothelial cells [65–70] with subsequent activation of NF-κB and p38 MAPK, and suppression of vasculo-protective Krüppel-like factors [71–73]. Modulation of these pathways leads to suppression of anti-inflammatory transcription factors, reduction in nitric oxide synthesis, and increased tissue factor synthesis [33, 34, 69, 70, 72–76]. In a mouse model, aPL increased tissue factor activity in carotid homogenates [77]. Meanwhile, aPL administration to mice also results in increased leukocyte-endothelium interactions [78, 79]. Concordantly, mice can be protected from aPL-mediated thrombosis by disrupting the function of E-selectin and P-selectin (the key selectins expressed on endothelium), P-selectin glycoprotein ligand-1 (**PSGL-1**, a key selectin ligand), or endothelial integrin ligands VCAM-1 and ICAM-1 [76, 79, 80].

Platelets. Platelets are being increasingly recognized for their roles that extend beyond hemostasis and thrombosis. Platelet-leukocyte interactions result in bidirectional immune crosstalk and transactivation, with downstream

Table 2 Some mechanistic highlights of APS pathophysiology

Cell or pathway	In vitro, aPL…	In patients, we can find…
Endothelial cells	Increase expression of tissue factor and adhesion molecules [33, 34]	More endothelium-derived microparticles [35, 36]
Platelets	Induce activation under shear stress [37]	Increased platelet-leukocyte aggregates [38]
Monocytes	Trigger expression of tissue factor [39–42] and pro-inflammatory cytokines [43–45]	Increased tissue factor-expressing monocytes [46–48]
Neutrophils	Promote release of prothrombotic neutrophil extracellular traps (NETs) [49]	High levels of circulating NETs [49] and anti-NET antibodies [50]
Complement	Trigger cell lysis as measured by modified Ham test [51]	High levels of complement split products [52–54]
Coagulation	Interfere with coagulation inhibitors, especially protein C and antithrombin [55, 56]	High levels of the active free thiol form of factor XI [57]
Fibrinolysis	Interfere with activity of tissue plasminogen activator [58]	High levels of plasminogen activator inhibitor-1 (PAI-1) [59–61]

effects on vascular inflammation. Circulating platelet-leukocyte aggregates are detected at increased levels in patients with APS, consistent with persistent, low-grade platelet activation [38]. The unstimulated platelet surface resists binding by β_2GPI protein and anti-β_2GPI antibodies; however, under shear stress, β_2GPI engages surface ApoER2 and GPIb, creating a platform by which anti-β_2GPI antibodies can then trigger platelet activation [37]. Meanwhile, aPL also activate platelets primed by low levels of thrombin in a mitogen-activated protein kinase (MAP kinase)-dependent fashion [81]. In a mouse model of APS, aPL-activated platelets are preferentially recruited to injured endothelium where they are required for fibrin generation in the expanding thrombus [82]. As discussed above, thrombocytopenia commonly complicates the course of APS. The extent to which this thrombocytopenia of APS is attributable to low-grade platelet activation and subsequent clearance, or to autoimmune-mediated removal via anti-platelet glycoprotein antibodies likely varies from patient to patient [83–86].

Monocytes. The relative ease of monocyte isolation from peripheral blood has led to deeper characterization of monocytes than with endothelial cells or platelets. For example, it was demonstrated 20 years ago that in patients with lupus, the presence of aPL is associated with enhanced monocyte tissue factor production [87]. Similar findings have been appreciated in patients with primary APS [46–48]. Beyond tissue factor, APS monocytes also express high levels of VEGF and its receptor Flt-1 [88]. Unbiased transcriptomic profiling has demonstrated upregulation of proinflammatory genes including *TLR8*, *CD14*, and genes associated with oxidative stress [89, 90]. APS monocytes have also been shown to upregulate certain protease-activated receptors [91], best known for their response to activated coagulation factors such as thrombin but also now appreciated for their immune signaling functions. Monocyte-derived microparticles are found at increased levels in APS circulation [92, 93], where they are potentially an important source of tissue factor [36].

Experimentally, aPL trigger monocytes to express tissue factor [39–42] and pro-inflammatory cytokines including TNF-α and IL-1β in vitro [43–45]. Concordantly, neutralizing tissue factor in mice with a blocking antibody protects against aPL-mediated venous thrombosis [94]. Although circulating monocytes have not, for the most part, been specifically characterized in animal studies, one interesting report demonstrated that the introduction of a *Nox2* (NADPH oxidase) mutation into bone marrow-derived cells (e.g., myeloid cells but not endothelial cells) protects against venous thrombosis [94].

Neutrophils. Neutrophils are the most abundant leukocytes in circulation where they patrol the bloodstream waiting to be recruited to sites of inflammation. Until recently, phagocytosis was thought to be the dominant mechanism by which neutrophils neutralized invading pathogens [95, 96]. In 2004, Brinkmann and colleagues described a process whereby neutrophils eject webs of chromatin into the extracellular space [97, 98]. These neutrophil extracellular traps (**NETs**) are tangles of decondensed extracellular DNA and histones decorated with microbicidal proteins derived from neutrophil granules and cytoplasm. NETs are released in response to both infectious and sterile stimuli including bacteria, fungi, protozoa, and viruses, as well as activated platelets and endothelial cells, complement proteins, cytokines, autoantibodies, and immune complexes [99]. While NETs likely evolved to trap pathogens, they are also now well recognized to be prothrombotic (Fig. 1) [100]. NETs activate platelets and clotting factors and can be found in both deep vein thrombi [101–104] and arterial clots [105–107]. Indeed, studies by various groups have shown that disrupting neutrophil-endothelium interactions, preventing NET formation, and dissolving NETs are all strategies that can mitigate thrombosis in animal models [104, 108–115].

In the 1990s, prior to the first descriptions of NETs, it was found that mouse monoclonal antibodies against human β_2GPI activated neutrophils, stimulating degranulation and hydrogen peroxide production [116]. In the early 2000s, an important series of experiments characterized pregnancy models of APS and found that neutrophils and complement were important mediators of fetal injury [117–119]. In vitro experiments have demonstrated that various human monoclonal aPL induce neutrophil activation as measured by oxidative burst, phagocytosis, and shedding of L-selectin [120]; these phenotypes were potentiated by lipopolysaccharide and Pam3Cys-Ser-(Lys)4, demonstrating the potential for synergy with Toll-like receptor signaling [120]. Another study found increased tissue factor expression by control neutrophils cultured with APS serum; in this system, complement activation, and specifically the C5a receptor, was required for maximum tissue factor expression [121].

In one of the first studies to evaluate a potential role for NETs in APS, pre-formed NETs were exposed to APS patient serum [122]. As compared with healthy serum, the authors found that approximately 13% of APS serum samples (both primary and secondary) were defective in NET degradation [122]. In the same study, "anti-NET antibodies" were detected by adding APS serum to preformed NETs and then visualizing IgG deposition [122]. The concept of anti-NET antibodies has been more comprehensively examined in a recent study [50]. In a cohort of 76 patients with primary APS, the authors found IgG and IgM anti-NET antibodies to be markedly elevated as compared with healthy controls. Anti-NET antibodies did not correlate with anti-β_2GPI antibodies but did associate with impaired NET degradation by patient serum as well as a clinical history of recurrent venous thrombosis [50]. The extent to which anti-NET antibodies recognize similar antigens as the anti-chromatin antibodies

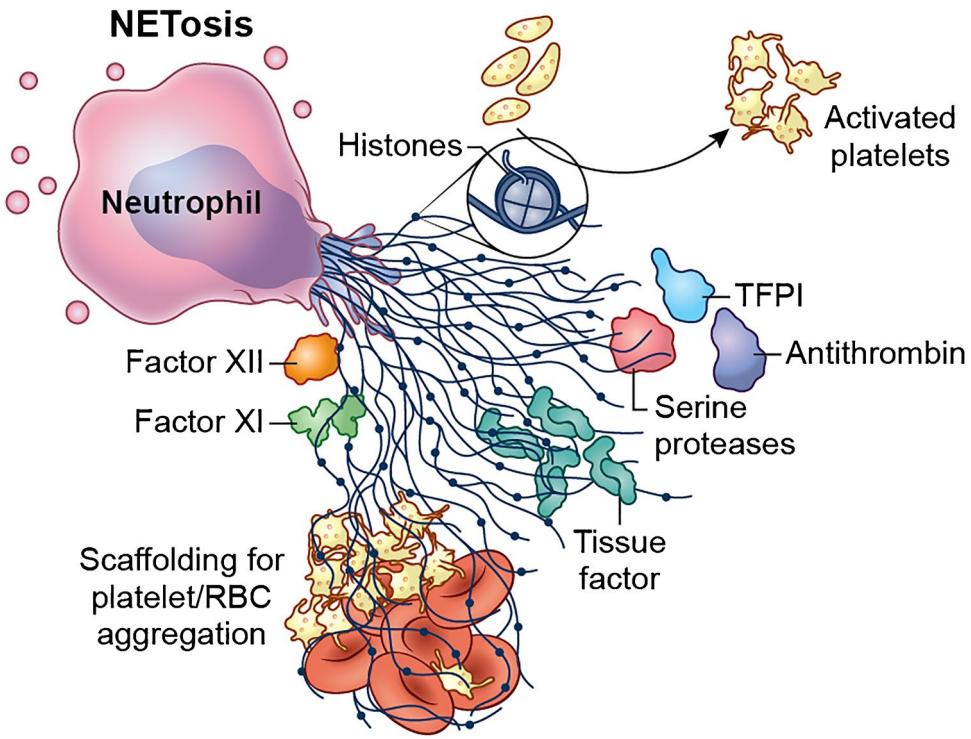

Fig. 1 Neutrophil extracellular promote thrombosis. Activated neutrophils release decondensed chromatin decorated with nuclear (histones), granule (proteases that degrade antithrombotic molecules such as TFPI and antithrombin), and cytoplasmic proteins that promote inflammation and coagulation (tissue factor, factor XI and XII). Together, NETs form a scaffold for cell aggregation and thrombus formation. TFPI = tissue factor pathway inhibitor. Illustration credit: Ethan Tyler (NIH)

previously described in primary APS is an intriguing question worthy of further study [123].

A study in 2015 was the first to show an association between aPL and NET release [49], a finding that has since been replicated by independent groups [124, 125]. The authors of the original study found high levels of NETs in circulation even in the absence of active thrombosis and that freshly isolated neutrophils from patients with APS released more NETs than neutrophils from healthy patients [49]. Moreover, human monoclonal anti-β_2GPI antibodies promoted NET release, while patients with triple-positive APS (presence of anticardiolipin, anti-β_2GPI, and lupus anticoagulant) tended to have the highest levels of circulating NET remnants [49]. Mechanistically, aPL-stimulated NET release depended on ROS generation by the NADPH oxidase and TLR4 signaling [49], with a role for Mac-1-mediated adhesion [126]. In vivo experiments used a flow restriction model of venous thrombosis to characterize aPL-mediated thrombosis in mice [127]. Mice administered APS IgG formed large thrombi that were enriched for NETs [127]. Meanwhile, both neutrophil depletion and deoxyribonuclease administration reduced thrombosis in APS mice to levels observed in control mice [127]. Other NET-disrupting strategies that mitigate aPL-mediated thrombosis in mouse models include PSGL-1 deficiency or inhibition [79], activation of cell surface adenosine receptors by drugs such as dipyridamole and defibrotide [128, 129], and even administration of ginger-derived phenolic substances, which function as phosphodiesterase inhibitors [130]. In patients, administration of the antioxidant coenzyme Q10 has been suggested as a complementary strategy for inhibiting NETs [131, 132].

Low-density granulocytes (LDGs), a subset of neutrophils best characterized in lupus, are proinflammatory and have a low threshold for releasing NETs [133–137]. van den Hoogen and colleagues recently found higher frequency of LDGs in APS patients whether or not the patients had coexisting lupus [138]. Notably, they also observed that anti-β_2GPI-positivity was predictive of APS patients who would have more LDGs in circulation.

A few studies have examined gene expression in APS neutrophils. In one, transcriptomic analysis of APS neutrophils by RNA sequencing revealed increase expression of pro-inflammatory genes, particularly with regard to type I interferon signaling, Toll-like receptor signaling, and metabolic reprogramming [79]. *IFIT1*, a type I interferon responsive gene, was most significantly upregulated (8.5-fold) in APS neutrophils [79]. In another study, genome-wide DNA methylation analysis of APS neutrophils did not find notable demethylation of interferon genes as has been previously reported for lupus neutrophils, suggesting divergent

epigenomic signatures [139]. Gene ontology analysis of hypomethylated genes in APS neutrophils demonstrated an enrichment of *ETS1*, *EMP2*, *OXT*, and *DPPA3*, all genes associated with mammalian pregnancy [139]. The physiologic consequences of epigenetic changes in APS neutrophils, including their potential relevance to APS-associated pregnancy morbidity, remain to be elucidated.

Taken together, these studies suggest exaggerated NET formation and impaired NET degradation in APS, both mechanisms that could amplify the impact of NETs on thrombosis. However, numerous questions remain, including the extent to which NETs might contribute to obstetric and extra-criteria manifestations of APS. Given that β_2GPI is a recognized DNA-binding protein, future studies may also ask whether anti-β_2GPI antibodies provide the possibility for epitope expansion to traditional lupus autoantigens such as double-stranded DNA and chromatin in some patients [140].

Complement. Complement is a system of over 50 proteins of the innate immune system that interact via protease activity to promote inflammatory cell recruitment, opsonization and clearance of pathogens, and sometimes cell death. Complement also links inflammatory responses to coagulation pathways [141]. The system can be activated by different stimuli with eventual convergence at the level of C5a generation (a chemotactic and pro-inflammatory protein) and assembly of the so-called membrane-attack complex (inclusive of C5b, C6, C7, C8, and C9) [142].

There is evidence of smoldering complement activation in APS [52–54], via both the alternative pathway [143–145] and the classical pathway [146–148]. Mechanistically, an important recent study used sera and purified anti-β_2GPI antibodies to demonstrate C5b-9 deposition and complement-mediated cell death via what the authors described as a "modified Ham test" [51]; importantly, complement activation as measured by this novel test correlated clinically with both triple-positive status and recurrent thrombosis.

Animal models provide strong evidence linking the complement system to APS. After early work demonstrated that antagonizing complement could protect against pregnancy loss [149], attention turned to its potential role in aPL-accelerated thrombosis. In a femoral vein injury model of thrombosis, disrupting complement C3, C5, and C6 were all individually protective against thrombosis [150–153]. Similarly, antagonizing either C5 or C6 was protective in a mesenteric thrombosis model triggered by lipopolysaccharide [154]. A deeper understanding of the complement pathway in APS is now needed, including mechanisms by which it integrates inflammation and coagulation.

Clinical reports of the complement inhibitor eculizumab effectively treating thrombosis in APS and CAPS [155, 156] are intriguing. Although there is a paucity of therapeutics available to mitigate the high mortality associated with CAPS, well-designed, randomized clinical trials are needed to develop a stronger evidence basis for complement inhibition and appropriate patient selection in APS.

Coagulation. The complex of β_2GPI and anti-β_2GPI disrupts the annexin A5 "anticoagulant shield" whereby annexin A5 normally binds to and neutralizes procoagulant phospholipids such as phosphatidylserine on cell surfaces [157–159]. In addition, anti-β_2GPI antibodies have been reported to impair the natural ability of β_2GPI to blunt von Willebrand factor-dependent platelet aggregation [160].

As discussed above, aPL represent a broader repertoire of antigenic targets than β_2GPI and cardiolipin, and the effects of aPL on specific components of the coagulation system remain an area of investigation. For example, aPL contribute to so-called activated protein C resistance which occurs when activated protein C is unable to inactivate coagulation factors V and VIII [55, 161]. Some aPL have been found to antagonize antithrombin activity by inhibiting the heparin binding that is required for full activation of antithrombin [56]; meanwhile, aPL with activity against thrombin may further protect thrombin from inactivation by antithrombin [162]. Similarly, aPL targeting factors IX [163] and X [164] appear to prevent their negative regulation by antithrombin. Elevated levels of factor XI are a known risk factor for thrombosis in the general population [165], and as compared with age- and sex-matched controls, APS patients carry higher-than-expected circulating levels of the active free thiol form of factor XI [57]. The activity of tissue factor may also be potentiated in APS via aPL-mediated inhibition of tissue factor pathway inhibitor (TFPI), or disassembly of a normally inhibited TF complex at the cell surface [166–168].

Fibrinolysis. Impaired fibrinolysis has been found in APS patients with thrombotic as well as obstetric manifestations [169]. Some aPL may inhibit fibrinolysis by neutralizing the ability of β_2GPI to stimulate tissue plasminogen activator (tPA)-mediated plasminogen activation and fibrinolysis [58]. Furthermore, there are reports of APS-associated autoantibodies that directly antagonize various pro-fibrinolytic factors (e.g., anti-annexin-A2, anti-tissue-type plasminogen activator/**tPA**, anti-plasmin) [169–171]. Small studies have also demonstrated upregulation of natural anti-fibrinolytic proteins [59–61], most notably plasminogen activator inhibitor-1 (**PAI-1**, the physiologic inhibitor of both tPA, and urokinase plasminogen activator). Mechanistically, PAI-1 is upregulated in human umbilical vein endothelial cells upon exposure to anti-β_2GPI antibodies from APS patients [172]. Interestingly, PAI-1 appears to have diverse functions beyond its role in restraining fibrinolysis, as elevated PAI-1 levels have regularly been associated with chronic disease states including fibrosis (of lung, liver, and kidney) and atherosclerosis [173–176] raising the possibility that PAI-1 might also play a role in the chronic occlusive APS vasculopathy that will be discussed below.

Table 3 Classification criteria for catastrophic antiphospholipid syndrome (CAPS) [10]

Criteria
1. Evidence of involvement of three or more organs, systems, and/or tissues
2. Development of manifestations simultaneously or in less than a week
3. Confirmation by histopathology of small vessel occlusion in at least one organ or tissue
4. Laboratory confirmation of antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibodies, and/or anti-β_2GPI antibodies)
Definite CAPS requires all 4 criteria
Probable CAPS is based on any of the following:
All four criteria, except for only two organs, systems, and/or sites of tissue involvement
All four criteria, except for the laboratory confirmation at least six weeks apart due to the early death of a patient never previously tested for aPL
Criteria 1, 2, and 4
1, 3, and 4 and the development of a third event in more than a week but less than one month, despite anticoagulation

Catastrophic APS

CAPS is characterized by rapidly developing and widespread microvascular thrombosis causing ischemic injury (Table 3) [10].

Current standard of care treatment for CAPS includes anticoagulation (typically with unfractionated heparin), immunosuppression (with high-dose corticosteroids), and plasmapheresis to emergently reduce the circulating aPL burden [11]. Given the relative rarity of CAPS, few studies have had the opportunity to pursue deep, mechanistic studies. There is some suggestion of endothelial and/or platelet activation based on high levels of von Willebrand factor and P-selectin in circulation [177]. Complement activation has also been indirectly implicated in the pathogenesis of CAPS as complement regulatory gene variants have been found in 60% of patients, perhaps contributing to uncontrolled complement activation [51]. Most patients with CAPS have an identifiable precipitating event such as surgery, infection, or pregnancy, which may serve as a complement-stimulating "second hit" in the setting of germline variants that may have reduced capacity to restrain complement amplification.

The potential role of complement in CAPS is further supported by reports of successful use (as mentioned above) of eculizumab in patients refractory to standard therapies. One series reported that 5 of 11 patients with CAPS responded to treatment with eculizumab [156]; individuals who had a response were more likely to have microangiopathic hemolytic anemia and thrombocytopenia, while those who already had dialysis-dependent kidney failure were less likely to respond. In another report, eculizumab allowed successful kidney transplantation in two patients with CAPS [178]. Based on these and other reports, the enthusiasm in the clinic for complement-inhibiting approaches to CAPS remains high, and additional evidence for this is eagerly anticipated.

APS vasculopathy

Distinct from APS-associated thrombotic events that acutely close vessels, the chronic occlusive vasculopathy of APS is characterized by cell proliferation and infiltration that progressively expands the intima [179–181]. These lesions are reminiscent of those seen following vascular interventions such as angioplasty and stent deployment in which the intima becomes thickened due to proliferation of vascular smooth muscle cells and production of proteoglycan-rich extracellular matrix between the endothelium and the internal elastic lamina [182]. Although this pathology was initially reported—and is still best defined—in the small vessels of APS kidneys [183–186], occlusive APS vasculopathy has also been observed in small- and medium-sized vessels of the brain, heart, and mesentery [179–181].

The molecular pathways that license these lesions are for the most part unknown, although one yet-to-be-reproduced report posited that the mTOR/Akt pathway is an important mediator of APS nephropathy and therefore a potential pharmacologic target via clinically available agents such as sirolimus [187]. mTOR is a kinase that integrates a variety of signaling pathways to regulate cellular growth, proliferation, and survival. In individuals with aPL-associated nephropathy, the vascular endothelium of intrarenal vessels was found to display molecular markers consistent with activation of mTOR and downstream signaling [187]. Furthermore, patients with aPL-associated nephropathy who required transplantation and were receiving sirolimus had minimal recurrence of vascular lesions, which contrasted with matched patients with aPL who were not receiving sirolimus [187].

The signaling pathways by which aPL trigger neointimal hyperplasia and occlusive vasculopathy may occur through direct or indirect interactions with the endothelium and smooth muscle of the vessel wall. Endothelial activation by aPL may create a dysfunctional, nitric oxide-depleted state

in addition to facilitating leukocyte and platelet adhesion. Proliferation of endothelial and smooth muscle cells may be supported by mTOR signaling, complement activation, macrophage foam cell formation, canonical cell adhesion molecules, and repression of pro-resolving factors, among other possible mechanisms. As mechanisms and new treatment approaches are investigated for the acute and catastrophic complications of APS, it will be important to keep in view the chronic sequelae of multi-system vasculopathy that also leads to progressive organ dysfunction in patients with APS.

COVID-19

Like APS, coronavirus disease 2019 (COVID-19) is associated with a high incidence of thrombosis in arterial, venous, and microcirculatory vascular beds [188, 189]. Notably, studies of COVID-19 patient samples demonstrate some similarities with APS, including evidence for aberrant activation of neutrophils [190, 191], endothelial cells [192], platelets [193], and complement [194]. A report from early in the pandemic detected aPL in three patients with COVID-19 who experienced cerebrovascular accidents [195]. This was soon followed by a study of 56 hospitalized in whom lupus anticoagulant was detected in 25; five of the patients also had either anticardiolipin or anti-β_2GPI antibodies [196]. Studies in COVID-19 patients have detected both traditional aPL and various "non-criteria" aPL (anti-phosphatidylserine/prothrombin IgG and IgM as well as anticardiolipin and anti-β_2GPI IgA). Studies have demonstrated significant heterogeneity in terms of prevalence of aPL (some as high as 50%) and which aPL species are most detected [197–199]. At the present time, it is mostly unknown whether these are transient aPL, as have been reported in other viral infections [200], or persistent aPL that herald long-term thrombotic risk.

Most studies have not found a clear association of aPL with macrovascular thrombotic events in COVID-19. Furthermore, functional assays such as lupus anticoagulant should be interpreted with caution in severely ill patients due to potential confounding by high levels of C-reactive protein and administration of anticoagulation. Despite these caveats, the relationship between aPL and COVID-19 is an emerging area deserving of further research. There is some evidence that IgG fractions isolated from the serum of patients with COVID-19 with high titers of aPL have prothrombotic properties in vitro and in mice [199, 201]. Future studies are required to determine persistence of these antibodies and identify mechanistic connections that can further clarify the extent to which aPL-like antibodies in patients with COVID-19 mimic those seen in patients with traditional APS.

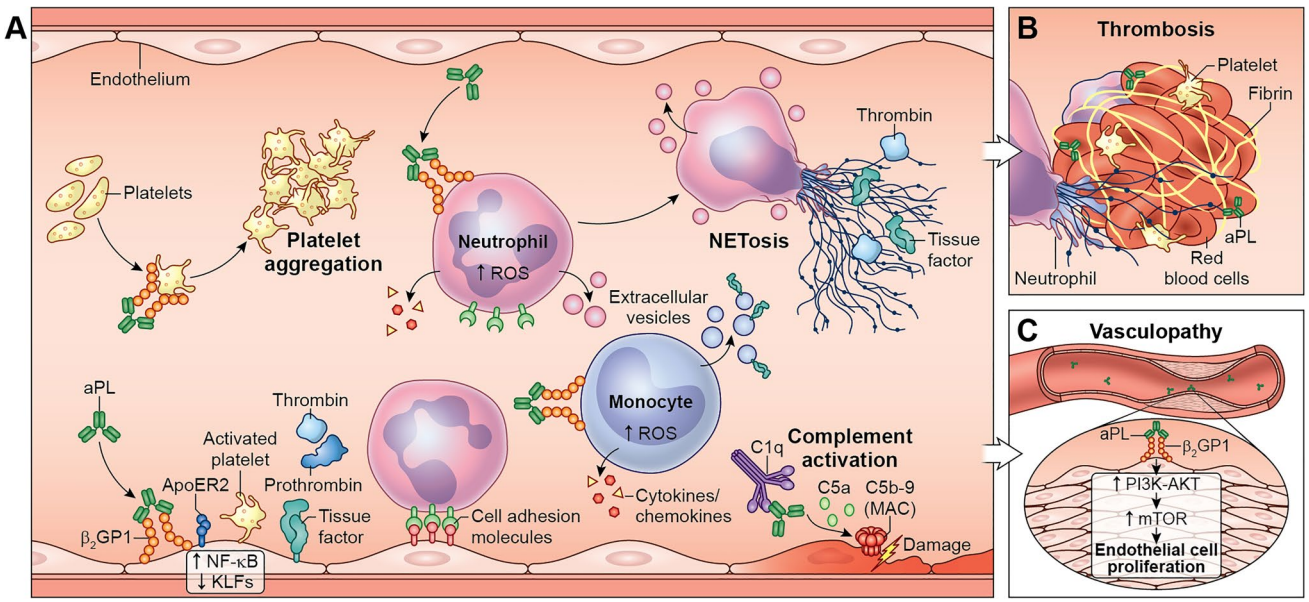

Fig. 2 Potential mechanisms contributing to thrombotic APS. **A** Endothelial cells increase expression of tissue factor (TF) and adhesion molecules. Complement damages the endothelium via the membrane attack complex (MAC) and acts as a chemoattractant via C5a. Monocytes express TF and cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and type I interferons (IFNs), and release microparticles. Neutrophils produce reactive oxygen species and release neutrophil extracellular traps (NETs). **B** NETs form an intravascular scaffold that promotes thrombus accretion. **C** Chronic activation of the endothelium by aPL can result in progressively occlusive vasculopathy. aPL = antiphospholipid antibodies; ApoER2 = apolipoprotein E receptor 2; β_2GPI = beta-2 glycoprotein I; NF-κB = nuclear factor kappa B; KLFs = Kruppel-like factors. Illustration credit: Ethan Tyler (NIH)

Summary

Like other systemic autoimmune diseases such as lupus, systemic sclerosis, and autoimmune vasculitis, there is significant person-to-person heterogeneity in individuals presenting with APS. One individual may present with heart valve lesions and thrombocytopenia, another with recurrent venous thrombosis, and another with livedo racemosa and white matter hyperintensities. The potential mechanisms covered above are myriad (Fig. 2) and the extent to which each mechanism manifests in a particular individual may help explain disease heterogeneity. One potential model is that aPL profiles are relatively consistent, while heterogeneity is best explained by comorbid genetic and acquired risk factors. Alternatively, aPL profiles may vary more than we realize as only a handful of types of aPL can be routinely tested for clinically. In that scenario, we will not be able to fully explain APS pathophysiology until the full autoantigenome of a particular individual has been defined; this is an important area for future research.

While the thrombophilia of COVID-19 does not appear to be explained by the best characterized aPL such as anti-β_2GPI antibodies, it does seem possible that less refined aPL-like antibodies do contribute to the COVID thrombotic burden, especially in the microvasculature. The ongoing global pandemic emphasizes the importance of more deeply defining understudied disease states such as APS. Meanwhile, the hope is that the relative spotlight APS has received during the pandemic will build momentum for significant discovery over the next decade in pursuit of the personalized proactive approaches to diagnosis and treatment that our patients deserve.

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Declarations

Conflict of interest JSK received support from Jazz Pharmaceuticals for preclinical research. YK is an inventor on a pending patent (US20180369278A1) filed by the University of Michigan on the use of biogases in vascular disease.

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