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Antiphospholipid syndrome: an update for clinicians and scientists

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Abstract

Purpose of review—Antiphospholipid syndrome (APS) is a leading acquired cause of thrombosis and pregnancy loss. Upon diagnosis (which is not made until at least one morbid event has occurred), anticoagulant medications are typically prescribed in an attempt to prevent future events. This approach is not uniformly effective and does not prevent associated autoimmune and inflammatory complications. The goal of this review is to update clinicians and scientists on mechanistic and clinically-relevant studies from the past 18 months, which have especially focused on inflammatory aspects of APS pathophysiology.

Recent findings—How antiphospholipid antibodies leverage receptors and signaling pathways to activate cells are being increasingly defined. While established mediators of disease pathogenesis (like endothelial cells and the complement system) continue to receive intensive study, emerging concepts (such as the role of neutrophils) are also receiving increasing attention. *In vivo* animal studies and small clinical trials are demonstrating how repurposed medications (hydroxychloroquine, statins, rivaroxaban) may have clinical benefit in APS, with these concepts importantly supported by mechanistic data.

Summary—As anticoagulant medications are not uniformly effective and do not comprehensively target the underlying pathophysiology of APS, there is a continued need to reveal the inflammatory aspects of APS, which may be modulated by novel and repurposed therapies.

Keywords

Antiphospholipid syndrome; thrombosis; pregnancy loss; endothelial cells; neutrophils; complement

Introduction

Vascular complications, including thrombotic events, are among the leading causes of morbidity and mortality in lupus. Antiphospholipid antibodies (aPL), a major driver of

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thrombosis risk, are present in up to one-third of lupus patients. When aPL are associated with certain clinical complications (either thrombotic or obstetric), a diagnosis of antiphospholipid syndrome (APS) is assigned (Table 1) [1]. Beyond lupus-associated APS, approximately half of APS cases will be diagnosed as a standalone syndrome (i.e., primary APS) [2].

APS is a leading acquired cause of thrombosis and pregnancy loss, with an estimated prevalence of 1 in 2,000 [3]. Framing this risk another way, aPL can be detected on the order of 10% of the time in the setting of certain events including pregnancy morbidity, stroke, myocardial infarction, and deep venous thrombosis (DVT) [4]. Emphasizing the systemic nature of APS, the diagnosis also portends risk for cytopenias (especially hemolytic anemia and thrombocytopenia), mitral and aortic valve lesions, seizure disorder, accelerated cognitive decline, and nephropathy in the form of thrombotic microangiopathy [5]. The approach to treatment is typically with anticoagulant drugs, which are not uniformly effective in preventing recurrent aPL-mediated thrombosis and pregnancy loss, and offer insufficient protection against the varied “non-criteria” manifestations of APS. Indeed, 44% of “triple-positive” APS patients (positive testing for anticardiolipin, anti-beta-2-glycoprotein I, *and* lupus anticoagulant) will develop recurrent thrombosis over a 10-year follow-up period (even with the majority being prescribed anticoagulants) [6]. Furthermore, at least 20% of obstetric APS patients have adverse outcomes in spite of therapy with aspirin and low-molecular-weight heparin [7].

Despite its high prevalence and potential for devastating morbidity, APS pathophysiology has yet to be fully defined. APS was historically viewed as a coagulation problem; however, clinical observations and basic science discoveries are increasingly highlighting a more multifaceted syndrome with an associated (and perhaps even central) inflammatory component [8]. Herein we will discuss recent discoveries over the past 18 months, which have continued to increase our understanding of APS pathophysiology. We will also discuss how this improved basic understanding may translate to new and repurposed therapeutics for APS (Table 2)

Cell activation and signaling pathways: new concepts

Understanding the cellular signaling pathways that mediate APS pathogenesis has remained somewhat elusive, at least partially the consequence of study heterogeneity. Studies have utilized different types of aPL (monoclonal vs. patient-derived; protein cofactor-dependent vs. -independent) and have focused on a variety of cellular targets (endothelial cells, platelets, monocytes, neutrophils, trophoblast cells, etc.).

Many (perhaps most) pathogenic antibodies in APS do not target phospholipids themselves, but rather phospholipid-binding protein cofactors. The best characterized of these cofactors is beta-2 glycoprotein I (β_2GPI), a lipid-binding protein present at high levels in plasma [22,23], albeit with largely unknown endogenous function. The mechanistic schema is that anti-β_2GPI antibodies potentiate thrombosis by engaging β_2GPI protein that has been recruited to cell surfaces—and thereby promote cell activation [24–26]. The mechanisms by which anti-β_2GPI antibodies activate cells have been recently reviewed [27], with roles

especially suggested for the cell surface proteins annexin A2, apolipoprotein E receptor 2 (ApoER2), Toll-like receptor 2 (TLR2), and TLR4 [27].

ApoER2 (also known as LDL receptor-related protein 8) is one receptor for β_2GPI (and consequently β_2GPI-dependent aPL) on monocytes, endothelial cells, and platelets. Indeed, in a 2011 study, Ramesh and colleagues demonstrated ApoER2$^{-/-}$ mice are relatively resistant to thrombosis when confronted with aPL [28]. More recently, it has been revealed that ApoER2 may play an important role in obstetric APS [29]. Specifically, Ulrich and colleagues demonstrated enhanced placental trophoblast cell proliferation and migration *in vitro* when aPL engage β_2GPI/ApoER2 complexes on the trophoblast cell surface [29]. Extending these studies to an *in vivo* model of aPL-mediated pregnancy loss, they demonstrated protection in ApoER2$^{-/-}$ mice [29]. In another recent study, Mineo and colleagues developed a monoclonal antibody against β_2GPI that prevents pathogenic aPL binding, thereby protecting against aPL-mediated cell activation [30]*. The antibody attenuated the association of β_2GPI with ApoER2, thereby normalizing endothelial and trophoblast cell function *in vitro*, as well as preventing thrombosis and fetal loss *in vivo* [30]*. Although further study is clearly needed, the intersection of aPL, β_2GPI, and ApoER2 warrants further investigation as a potential therapeutic target in patients.

Since neither β_2GPI itself, nor some β_2GPI "receptors" such as annexin A2, have a cytoplasmic domain to mediate signaling, there has been interest in additional partner proteins that may convey activating signals to the cytoplasm. On this front, particular attention has been given to the cell-surface TLRs, TLR2 and TLR4. In mouse models, TLR4 deletion protects against venous and arterial thrombosis in some [31–33], but not all [34]*, studies (it is worth pointing out that the latter study utilized cofactor-independent aPL). Studies of obstetric APS have also yielded mixed results with an older study demonstrating no role for TLR4 in an *in vivo* model of pregnancy loss [35]. In contrast, Azuma and colleagues recently suggested that, at least *in* vitro, TLR2 and TLR4 facilitate inflammatory cytokine production by trophoblast cells in response to anti-β_2GPI antibodies [36].

Signaling pathways downstream of the aforementioned receptors, at least as they relate to APS pathogenesis, remain incompletely understood. Terrisse and colleagues recently investigated downstream signaling pathways by which aPL (especially IgG isolated from APS patients) activate platelets [37]*. The authors demonstrated that aPL potentiate *ex vivo* platelet activation through surface glycoprotein Ibα (the platelet receptor for von Willebrand factor) and TLR2, via a mechanism involving class IA phosphoinositide 3-kinase (PI3K) α and β isoforms [37]*. At least one downstream consequence of PI3K signaling is activation of the serine/threonine kinase Akt, a pathway that supports cell survival, proliferation, and migration [37]*. Indeed, PI3K inhibitors, which are being explored as potential drug targets in other contexts [38], are effective at preventing aPL-mediated platelet activation [37]*. Interestingly, another study has suggested that Akt activation is a downstream consequence of trophoblast cell activation by aPL [29].

Beyond the engagement of aPL with cell surfaces, a recent report by Wu and colleagues suggests an intriguing new mechanism by which aPL-activated endothelial cells may propagate this activation in paracrine fashion to other endothelial cells [39]*. Anti-β_2GPI

antibodies trigger the release of “extracellular vesicles” from endothelial cells, which the authors define as inclusive of both microparticles and exosomes [39]*. These vesicles then activate endothelial cells through a mechanism that is not dependent upon packaged cytokines such as IL-1, but rather single-stranded RNA that signals through TLR7 in the recipient cell [39]*. They also speculate that these vesicles may be a mechanism for delivery of specific and functionally-relevant micro-RNA, although this hypothesis requires further study.

The vessel wall: endothelial progenitors and interferons

Our group recently looked “upstream” of endothelial cells, asking whether a deficiency in reparative, circulating endothelial progenitors might contribute to defective maintenance and health of the endothelium over time. Indeed, a deficiency in the number and function of such progenitors is a well-recognized aspect of both lupus and rheumatoid arthritis [40]. We found that primary APS patients have a reduction in functional endothelial progenitors, which was interestingly not dependent upon patient IgG; rather, we discovered a type I IFN signature in the APS patients, abrogation of which could restore normal progenitor function [41]*. These findings were recently replicated by van den Hoogen and colleagues, who found that approximately 50% of primary APS patients have a type I IFN signature, which was less likely to be present in patients taking either hydroxychloroquine or statins [20]**. Interestingly, they also found that the IFN signature correlated with expansion of “intermediate” and “non-classical” monocytes (which have been previously linked to cardiovascular disease in lupus and rheumatoid arthritis) [20]**. How these monocytes intersect with endothelial progenitors [42], and whether there is a role for anti-interferon therapy in APS [43], are questions that deserve further consideration.

One potential consequence of endothelial cell (and progenitor) dysfunction is atherosclerosis, an accelerated version of which is a well-known complication of lupus [44], and which has also been reported in APS [45,46]. The recent work of Benagiano and colleagues has examined the role of T_H1 specific inflammatory responses to β_2GPI in established atherosclerotic lesions of primary APS patients. Their work demonstrated that plaque-derived, β_2GPI-specific CD4+ T lymphocytes facilitate perforin- and Fas ligand-mediated cytotoxicity, pointing to a role for these autoreactive T cells in plaque destabilization (and potentially the arterial thrombotic events that are known to occur at higher frequency in APS) [47]**. They also demonstrated that β_2GPI can induce proliferation of (and IFN-γ expression by) plaque-derived T cell clones [47]**. Furthermore, these T cells amplify monocyte responses, such as the production of tissue factor and matrix metalloproteinases, which can be inhibited with an anti-IFN-γ antibody [47]**.

Myeloid-lineage cells: neutrophil extracellular traps (NETs) and monocyte NOX2

The role of neutrophils in APS pathogenesis has only recently been investigated. This interest was precipitated by emerging descriptions of neutrophils as mediators of both pathologic clotting and autoimmune diseases [48,49]. In particular, NETs (extracellular

chromatin-based structures released by activated neutrophils) have been described as triggers of autoimmunity and tissue damage, as well as important instigators of thrombosis [50].

With this background in mind [51], our group recently identified increased levels of cell-free DNA and NETs in the circulation of primary APS patients, as compared with healthy controls [52]**. When APS neutrophils were cultured *in vitro*, they demonstrated an enhanced propensity to spontaneously release NETs [52]**. Mechanistically, anti-β_2GPI IgG appears to be at least one factor in patient blood that supports NET release, with the mechanism dependent upon both TLR4 and formation of reactive oxygen species [52]**. Furthermore, the prothrombotic potential of aPL-mediated NETs was demonstrated in a thrombin generation assay, with this potential abrogated by treatment with deoxyribonuclease (DNase) [52]**. In parallel to our work, van den Hoogen and colleagues reported increased levels of circulating “low-density granulocytes” or LDGs in patients with primary APS [53]. This pro-inflammatory subset of neutrophils has been well characterized in SLE and other autoimmune disorders, where they are reported to release NETs in exaggerated fashion [54]. Whether LDGs are important sources of NETs in APS awaits further study [55].

The *in vivo* relevance of NETs was recently confirmed by our group in a mouse model of APS. In this model, IgG from triple-positive APS patients potentiated venous thrombosis in mice that had been subjected to flow restriction in the inferior vena cava by a standard surgical stenosis [56]*. As compared with control mice, mice treated with APS IgG were twice as likely to develop macroscopic thrombi in response to flow restriction. Mechanistically, APS thrombi were enriched for NETs, while patient IgG could be detected on the surface of circulating neutrophils [56]*. Furthermore, APS IgG-mediated thrombosis could be reversed by either neutrophil depletion or administration of systemic DNase [56]*. Around the same time, Manukyan and colleagues published an elegant study demonstrating that cofactor-independent aPL could similarly potentiate thrombosis in an inferior vena cava flow-restriction model [34]*. Their interesting work found a major role for leukocyte activation in thrombus formation, which could be abrogated by deletion of NOX2 (the catalytic subunit of NADPH oxidase) from bone marrow-derived cells. While the authors’ primary interest was in monocyte NOX2 and its role in tissue factor expression, there is also a well-accepted role for neutrophil NOX2 in NET formation [57]. Further studies may assess the role of these cofactor-independent antiphospholipid antibodies in inducing NET release *in vitro* and *in vivo*.

Complement: at the intersection of coagulation and inflammation in APS

Animal models of APS have supported a role for complement activation in both thrombotic events and pregnancy loss [58,59]. Studies in APS patients have demonstrated smoldering activity of the complement cascade [60–62], while a recent case report revealed deposition of β_2GPI protein, IgG, and complement components C1q, C4, C3, and C5b-9 at the endothelial surface of an occluded artery in an APS patient [63]. Furthermore, this patient, who had suffered recurrent arterial occlusions, was successfully revascularized while under treatment with eculizumab, a terminal complement inhibitor [63].

In lupus, antibodies to C1q (a complex that initiates the complement cascade in response to immune complexes) amplify complement activation and strongly correlate with certain clinical manifestations such as proliferative nephritis [64]. Oku and colleagues recently investigated these antibodies in primary APS patients, demonstrating that 36% of patients had detectable anti-C1q (compared to 55% of lupus patients) [65]. Interestingly titers of anti-C1q were significantly higher in patients with refractory APS [65].

Rivaroxaban, a direct factor Xa inhibitor, has recently been proposed as an alternative agent to vitamin K antagonists in APS. The first randomized, prospective study investigating use of rivaroxaban in APS (RAPS trial) was recently published. In patients with a history of venous thromboembolism (who had already demonstrated stable disease on warfarin), both warfarin and rivaroxaban prevented new thrombotic events for 210 days in every study patient [14]**. Bleeding events and overall adverse events were also similar between the groups [14]**. While a full recounting of this important trial is beyond the scope of this brief review, we would refer you to a detailed comment on the topic [66]. Related to our discussion of the complement pathway, a post-hoc analysis of the RAPS trial revealed that, prior to randomization, APS patients had significantly higher markers of complement activation as compared with normal controls [19]*. While patients in the warfarin group showed stable elevation of these markers over time, patients randomized to rivaroxaban demonstrated decreased C3a, C5a, and soluble C5b-9 (all markers of classical pathway activation) [19]*. In contrast, the alternative pathway marker, Bb, was unchanged with rivaroxaban treatment [19]*. Whether direct oral anticoagulants have additional anti-inflammatory properties is a topic that certainly warrants further study.

Repurposing medications: statins and hydroxychloroquine as adjuvant therapies in APS?

HMG-CoA reductase inhibitors (or statins) have long been recognized to have pleotropic anti-inflammatory effects supportive of vascular health, including reductions in inflammation, oxidative stress, and coagulation [67]. Clinically, statins appear to reduce the risk of venous thromboembolism in the general population [13]. In mouse models of APS, statins mitigate aPL-mediated thrombotic events and fetal death [11,16]. Furthermore, when administered to APS patients, statins decrease both prothrombotic and proinflammatory biomarkers [68].

The standard of care for managing pregnancy complications in APS is the administration of low-dose aspirin and low-molecular-weight heparin (the latter at either prophylactic or therapeutic doses, depending on the patient’s thrombosis history) [69,70]. However, as detailed in recent review articles [69,70], pregnancy complications in APS are often not based in frank placental thrombosis, but rather spiral artery vasculopathy, as well as acute and chronic inflammation—with increased infiltration of inflammatory cells and deposition of complement in the placentae of women with APS [71–73]. Lefkou and colleagues recently investigated the use of pravastatin in refractory obstetric APS [18]**. In their clinical trial, 21 patients with refractory obstetric APS (emergence of preeclampsia and/or intrauterine growth restriction [IUGR] despite treatment with low-dose aspirin and low-

molecular-weight heparin) were randomized either to continue standard therapy or to receive pravastatin 20 mg/day at the onset of preeclampsia/IUGR [18]**. There was a remarkable therapeutic benefit, with all the patients receiving pravastatin delivering healthy infants at 34–38 weeks [18]**. In contrast, the 10 patients who remained on standard therapy had three stillbirths at 25–26 weeks, and seven pre-term Cesarean sections (resulting in two fetal deaths) [18]**.

Hydroxychloroquine (which is nowadays prescribed to essentially all patients with lupus) was utilized in the 1970s to reduce the risk of venous-thromboembolism in post-operative patients [12]. In the 1990s, hydroxychloroquine was demonstrated to protect against aPL-mediated thrombosis in mice [9]. Furthermore, there have been hints of a reduction in thrombosis risk in lupus patients taking hydroxychloroquine, as compared to those who are not [74,75]. Mechanistically, a recent study demonstrated that hydroxychloroquine inhibits the translocation of monocyte NOX2 to the endosome in response to stimulants such as TNFα, IL-1β, and aPL [10]**. This was accompanied by mitigation of aPL-induced, NOX2-mediated thrombus formation *in vivo* [10]**. As the related drug chloroquine has been shown to antagonize NET release [21], further studies should continue to explore the intersection of hydroxychloroquine, activated monocytes/neutrophils, and APS.

Given its excellent safety profile in pregnancy [76], and its nearly standard-of-care application in lupus pregnancies, hydroxychloroquine has been increasingly considered as adjuvant therapy in APS pregnancies. Indeed, recent retrospective studies have suggested a beneficial effect of hydroxychloroquine in APS pregnancies [7,17]. In a mouse model of obstetric APS, Bertolaccini and colleagues recently demonstrated that hydroxychloroquine prevents fetal death and placental metabolic changes [15]*. Going further, they demonstrated that labeled aPL especially localize to the placenta and the developing fetal brain, and that hydroxychloroquine mitigates complement deposition at both sites (which correlated with lower levels of C3a and C5a in blood) [15]*. Intriguingly, C3a and C5a were also reduced in the blood of APS patients after 6 months of hydroxychloroquine treatment [15]*.

Conclusion

Since its description in the 1980s, APS has been managed primarily with anticoagulant medications. These medications are not universally protective against subsequent thrombotic events and pregnancy loss, and have little proven track record in treating “non-criteria” manifestations of APS such as cytopenias and cardiac valvular disease. Basic science studies continue to refine the signaling pathways, activated cells, and non-cellular effectors critical for APS pathogenesis (Figure 1). In addition to a search for novel therapeutics, established medications such as rivaroxaban, statins, and hydroxychloroquine are receiving increasing interest as adjuvant therapies. In the near future, we hope to see more well-designed clinical trials with both mechanistic and clinical endpoints.

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Key points

1. Current standard-of-care therapy for APS does not explicitly target inflammatory aspects of APS pathophysiology.
2. A better understanding of inter- and intra-cellular signaling pathways in APS has revealed potential drug targets (i.e., interferons, phosphoinositide 3-kinase, etc.).
3. In addition to the well-established cellular mediators of APS pathogenesis (endothelial cells, platelets, etc.), there is emerging interest in the contribution of myeloid-lineage cells to APS pathogenesis. The role of neutrophil extracellular trap release, in particular, warrants further study.
4. Complement activation and deposition continue to be recognized for their role in APS pathogenesis. Activity of this pathway may be mitigated by several medications including rivaroxaban and hydroxychloroquine.
5. Adjuvant therapeutics including statins and hydroxychloroquine have the potential to improve APS pregnancy outcomes, based upon animal studies and small clinical trials.

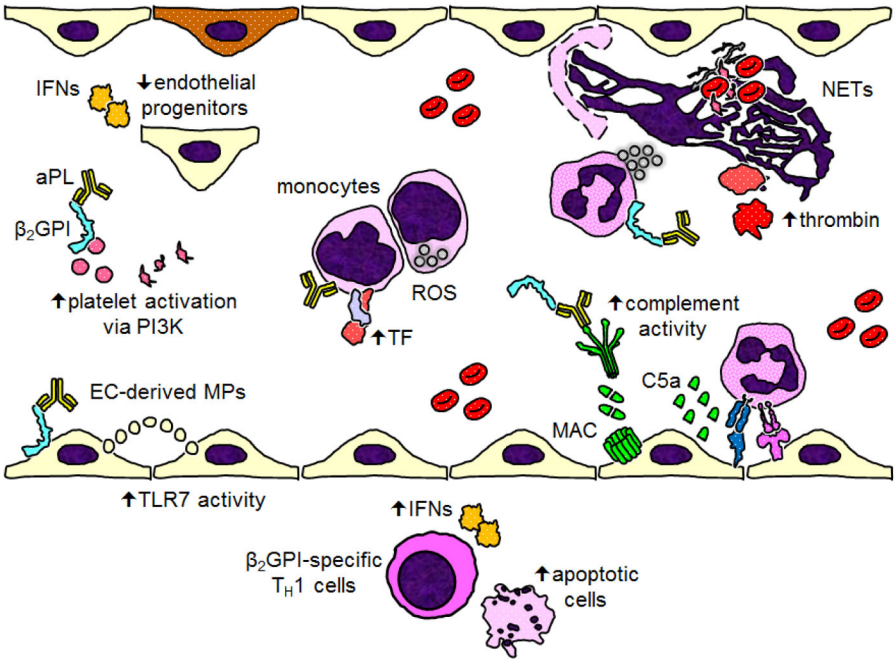

Figure 1.

Recent mechanistic insights into the pathophysiology of antiphospholipid antibodies (aPL) and APS. Starting at the bottom of the figure and moving roughly clockwise: In the vessel wall of atherosclerotic plaques, beta-2 glycoprotein I (β_2GPI)-specific T_H1 cells trigger cell death and release interferons (IFNs). Endothelial cells (ECs) release vesicles (like microparticles) that activate TLR7 in other ECs by delivery of single-stranded RNA. aPL-mediated platelet activation relies on phosphoinositide 3-kinase (PI3K). Type I IFNs reduce the function of restorative circulating endothelial progenitors, which may lead to the accrual of endothelial damage over time. Cofactor-independent aPL activate monocytes via endosomal reactive oxygen species (ROS), resulting in increased expression of tissue factor (TF). In response to aPL, neutrophils release neutrophil extracellular traps (NETs), which help facilitate thrombin activation. Complement activation, especially through the classical pathway, leads to the assembly of the membrane attack complex (MAC) on the endothelial surface, while also facilitating the recruitment and activation of inflammatory cells.

Table 1

Classification Criteria for Antiphospholipid Syndrome [1]

APS is present if 1 of the clinical criteria and 1 of the laboratory criteria are met

Clinical criteria	1. Vascular thrombosis	≥1 clinical episode of arterial, venous, or small-vessel thrombosis.
	2. 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 pre-eclampsia or eclampsia defined according to standard definition **ii.** Recognized features of placental insufficiency **c.** ≥3 unexplained consecutive miscarriages at <10 weeks 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 isoforms **c.** Medium- to high-titer anti-beta-2 glycoprotein-I (anti-β_2GPI) antibodies of IgG or IgM isoforms	

Table 2

Summary of efficacy and mechanisms by which adjuvant therapeutics could potentially benefit APS patients

		Hydroxychloroquine	Statins	Rivaroxaban
Summary of efficacy:				
Thrombotic risk	Mouse models	Protects [9,10]	Protects [11]	
	APS patients	No prospective studies in APS, but protects in post-operative setting [12]	No studies in APS, but protects in the general population [13]	Efficacy may be similar to warfarin (although further study is needed) [14]
Obstetric events	Mouse models	Prevents fetal death and metabolic changes [15]	Prevents fetal death [16]	
	APS patients	May prevent pregnancy loss [7,17]	May prevent fetal morbidity and mortality [18]	
Potential anti-inflammatory mechanisms:				
Complement		Inhibits activation and deposition [15]		Decreases activation [19]
Type I IFN signature		Decreases [20]	Decreases [20]	
NET release		Possibly inhibits [21]		