Published in final edited form as:

Curr Opin Rheumatol. 2023 March 01; 35(2): 89–97. doi:10.1097/BOR.0000000000000926.

An update on inflammation in antiphospholipid syndrome (APS)

Amala Ambati[1], **Yu Zuo**[1], **Jason S. Knight**[1]

[1]Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA

Abstract

Purpose of review: Antiphospholipid syndrome (APS) is an acquired thrombo-inflammatory disease associated with diverse clinical manifestations in the setting of persistently circulating antiphospholipid antibodies (aPL). This review summarizes recent developments in our understanding of the pathogenesis of APS and its various clinical manifestations with a focus on the activation of endothelial cells, complement, and neutrophils.

Recent findings: Elucidating the pathophysiology that leads to the diverse array of clinical manifestations of APS is an area of active exploration. Here, we highlight recent studies that have explored various impacts of endothelial activation and injury in APS, including the promotion of circulating endothelial cells and extracellular vesicles; the association between complement activity and different APS phenotypes, including pregnancy loss; and the relationship between neutrophil extracellular traps (NETs) and high-risk aPL profiles in thrombotic APS. We also call attention to recent work that proposes approaches to mitigating these pathologic changes as potential treatment strategies for APS. Lastly, we highlight promising future directions in APS research such as multi-omics approaches to molecularly stratifying APS patients.

Summary: The identification of novel aspects of pathogenesis and more nuanced approaches to phenotyping patients will hopefully pave the way for developing safer and more effective patient-specific therapeutic strategies for APS.

Keywords

antiphospholipid syndrome; antiphospholipid antibodies; inflammation; complement; neutrophil extracellular traps

INTRODUCTION

Antiphospholipid syndrome (**APS**) is an acquired thrombo-inflammatory autoimmune disease with various potential clinical manifestations, including arterial, venous, and microvascular thrombosis, as well as obstetric morbidity (1, 2). Current classification criteria for APS require the presence of both clinical events and positive laboratory tests. APS is confirmed when there is a history of either vascular thrombosis (arterial, venous,

Correspondence: Jason S. Knight, MD, PhD, 1150 W. Medical Center Dr., Ann Arbor, MI 48109, jsknight@umich.edu.

CONFLICTS OF INTEREST

The authors have no relevant financial conflicts of interest to disclose.

or microvascular) or obstetric morbidity (recurrent early miscarriage, fetal demise, or premature delivery due to severe preeclampsia or placental insufficiency) in the setting of one or more positive antiphospholipid antibody tests. The latter must be documented on two or more occasions at least 12 weeks apart. Antiphospholipid antibodies (**aPL**) may be detected by one of three tests, including lupus anticoagulant (**LA**, a functional assay), anticardiolipin antibodies (**aCL**, by immunoassay), and anti-beta-2 glycoprotein-I antibodies (**aβ_2GPI**, also by immunoassay) (3). More recently, so-called "non-criteria" aPL, which are not part of current classification criteria, have demonstrated potential value when risk stratifying APS patients. Examples include IgG or IgM antibodies targeting phosphatidylserine/prothrombin complexes, IgA antibodies against cardiolipin or β_2GPI, or IgG antibodies targeting lysobisphosphatidic acid complexed with the endothelial protein C receptor (4). However, one must keep in mind that the clinical significance of these non-criteria aPL have not been completely elucidated, and the assays used to identify them are not yet fully standardized. Clinical manifestations, not part of current classification criteria that clearly associate with APS include livedo reticularis/racemosa, skin ulcers, hematologic derangements (hemolytic anemia, thrombocytopenia), cardiac valve disease, nephropathy, and cognitive dysfunction (1). In a recent population-based study conducted in 2019, the estimated overall prevalence of APS was 50 per 100,000 people, and the incidence was approximately two persons per 100,000 people per year (5).

Arterial involvement in APS may include thrombosis in the cerebral vasculature leading to transient ischemic attack or stroke; alternatively, thrombosis may involve the coronary, renal, mesenteric, retinal, or other arteries leading to downstream end-organ damage such as myocardial infarction, nephropathy, or vision loss (6). The venous involvement of APS most commonly presents as thrombosis in the deep veins of the lower extremities; however, other venous sites, such as the lungs or liver, can also be involved (6, 7). Microvascular manifestations of APS present as thrombosis in the small vessels, often associated with a particular organ such as kidneys or skin. When microvascular thrombi involve multiple organ systems simultaneously, leading to multiorgan failure, catastrophic APS (**CAPS**) is diagnosed (8). As evidenced by the classification criteria and other disease manifestations described above, APS is a heterogenous disease driven by underlying thrombo-inflammatory processes. Here we aim to summarize the existing literature on the intersection of inflammation and vasculopathy in APS pathogenesis.

ENDOTHELIAL ACTIVATION AND INJURY

Given that many clinical manifestations of APS occur primarily in the intravascular space, it is essential to understand the mechanisms that tip the endothelium away from homeostasis and toward thrombosis. Thus far, what is known about endothelial changes in APS suggests a multifactorial process involving the activation of endothelial cells, downregulation of vasoprotective factors, and, in some cases, increased endothelial proliferation. *In vitro* studies have shown the ability of cell-surface β_2GPI/aβ_2GPI complexes to engage a variety of receptors, including apolipoprotein E receptor 2 (apoER2) (9, 10), annexin A2, and TLR4, which have the potential to tip the endothelium toward thrombosis (11). For example, the calcium-dependent phospholipid-binding protein annexin A2 supports the recruitment of β_2GPI/aβ_2GPI to the endothelial surface, where cell activation is triggered (12, 13). Indeed,

annexin A2-deficient mice had significantly smaller mean thrombus size and substantially less tissue factor activity when exposed to APS IgG or monoclonal aβ_2GPI in the setting of vascular injury (14). Downstream of surface receptors, the effects of aPL are thought to involve activation of p38 MAPK and NF-κB, as well as suppression of Kruppel-like transcription factors (15). Modulating these pathways can lead to the downregulation of vasoprotective nitric oxide, and the upregulation of tissue factor along with various cell adhesion molecules, which promote endothelial activation and thrombosis (15–17). Multiple APS animal studies have shown protection against thrombosis by blocking surface adhesion molecules such as E-selectin, P-selectin, VCAM-1, and ICAM-1 (18, 19).

Endothelial proliferation in APS results in what is referred to as neointima formation or intimal hyperplasia. It is thought to contribute to organ deterioration over time via the occlusion of smaller blood vessels. This phenomenon is best described in the kidneys (20–22) but is likely also present in other organs such as the skin, brain, and heart (23–25). The mammalian target of rapamycin (mTOR) is a kinase that is involved in the regulation of cellular growth and proliferation (26). A 2014 study utilized immunostaining to demonstrate that patients with APS-associated nephropathy showed evidence of activation of the mTOR pathway. This study also found that IgG antibodies from patients with antiphospholipid syndrome simulated the mTOR pathway in cultured vascular endothelial cells (27). A more recent study from 2022 found that skin biopsies of aPL-positive patients (with or without SLE) with livedo reticularis/racemosa had increased mTOR activity compared to skin samples of aPL-negative patients. Interestingly, the most prominent mTOR activity was observed not in blood vessels, but in the lower basal layers of the epidermis (28).

Recent work has also investigated the implications of circulating endothelial cells (CECs), extracellular vesicles, and exosomes in APS. The stressed or injured endothelium releases CECs and extracellular vesicles, both of which are elevated in APS (29, 30). A 2022 study quantified CECs from the blood of patients with aPL and/or SLE and found that patients with a history of obesity, myocardial infarction, venous thromboembolism, or nephropathy had more CECs in circulation (31). Additionally, the study also showed a significant correlation between CECs and other markers likely to be associated with endothelial dysfunction, including tissue factor-bearing extracellular vesicles and soluble TREM-1 (triggering receptor expressed on myeloid cells-1) (31). Another recent study isolated circulating exosomes from the plasma of pregnant patients with APS. These APS exosomes increased fetal resorption in a mouse model. They disrupted migration and tube formation by endothelial cells *in vitro* (32), the latter dependent on uptake of the exosomes by cultured endothelial cells. Interestingly, proteomic analysis of the APS exosomes demonstrated 27 upregulated proteins compared with control exosomes, the most notable of which was β_2GPI (32). The implications of this last finding remain to be fully elucidated.

Another collection of recent studies evaluated endothelial activation and dysfunction in cultured endothelial cells exposed to various serum factors from APS patients (33–35). One study found that IgG isolated from patients with different disease phenotypes (primary APS, secondary APS, APS refractory to treatment, and obstetric APS) resulted in the differential expression of markers related to endothelial activation and dysfunction (33). For example, polyclonal IgG from refractory APS patients increased the production of

microparticles, reactive oxygen species (**ROS**), and endothelin-1 while reducing nitric oxide production. In contrast, IgG from other types of patients showed different patterns (33). Another study evaluated the response of human umbilical vein endothelial cells (**HUVECs**) upon exposure to polyclonal IgG isolated from the serum of women with both pregnancy morbidity and vascular thrombosis (34). HUVECs were found to have increased cellular stress, as evidenced by increased mitochondrial hyperpolarization and increased activation of the mTOR and autophagic pathways (34). A third study showed that a complex of oxidized low-density lipoprotein/β_2GPI/aβ_2GPI contributes to endothelial dysfunction by inhibited autophagy via PI3K/AKT/mTOR and endothelial nitric oxide synthase signaling pathways (35).

Two recent studies have also shed light on potential treatment modalities that could aid in the prevention of endothelial activation and injury in APS (36, 37). The first study evaluated the effects of hydroxychloroquine on HUVECs exposed to polyclonal antibody fractions from pregnant APS patients (36). While APS IgG increased the expression of VEGF and matrix metalloprotinease-2 and decreased endothelial cell proliferation, migration, and angiogenesis, all of these phenotypes were reversed in the presence of hydroxychloroquine (36). Another study evaluated the effects of a heparinase inhibitor (RDS3337) on HUVECs incubated with affinity-purified aβ_2GPI (37). While aβ_2GPI treatment triggered interleukin receptor-associated kinase 1 (IRAK1) phosphorylation, NK-κB activation, and tissue factor expression, these phenotypes could be prevented by strengthening the glycocalyx with the heparinase inhibitor (37).

Notable and/or recent insights regarding endothelial cell activation in APS are highlighted in Table 1. Going forward, we hope to see more work on drivers and mechanisms of intimal hyperplasia in APS, as this likely underlies some of the most difficult-to-treat manifestations of APS in the clinic such as nephropathy and cognitive dysfunction. Available data are also mixed on what is the most crucial surface receptor for aPL on endothelial cells, which may relate to total IgG fractions being employed in most studies; indeed, IgG from COVID-19 patients behaves similarly to what would be expected from an individual with APS (38). Future studies will hopefully do more with affinity-purified antibody fractions as well as species beyond IgG in pursuit of understanding the extent to which endothelial cell-activating antibodies define a particular individual's clinical phenotype.

COMPLEMENT ACTIVATION AND COMPLEMENT-MEDIATED INJURY

The complement system is a critical mediator of innate immune responses and lays the foundation for adaptive immunity to mount a more specific and sustained defense. The system consists of proteins that activate one another through proteolytic cleavage via overlapping but distinct pathways (e.g., the classical pathway versus the alternative pathway). Activation of the complement cascade can have downstream proinflammatory effects, including cell lysis and the recruitment of inflammatory cells and other mediators (39). Complement activation has also been shown to potentiate the coagulation cascade through the C5a receptor, leading to endothelial activation and increased release of tissue factor (40). Early research regarding complement in the context of APS showed that it was an essential factor in the context of aPL-mediated pregnancy loss in animal models (41).

Subsequent studies found that mice with deficiencies in complement components such as C3, C5, or C6 were afforded at least some protection from the thrombophilia and vascular injury induced by aPL (42–44). Beyond engagement and activation of the endothelium, the complement system in APS is also likely to contribute to neutrophil activation as will be discussed in more detail in the next section (40, 45).

An interesting recent study explored the relationship between total C4b-binding protein (C4BPt; an inhibitor of the classical and lectin complement cascades), warfarin, and aPL, and found that C4BPt was significantly reduced in persistently aPL-positive patients, which could not be predicted by the presence of SLE, specific clinical manifestations, or the aPL profile (46). C4BPt demonstrated a negative association with complement activation products (such as C3dg). Through statistical inference via mediation analysis the study also found that a sizable percentage of the reduction in C4BPt was attributable to warfarin administration (46). Another recent study investigated the clinical significance of complement activation products C5a and C5b-9 amongst APS patients with quiescent disease (47). The patients were placed into three groups: those who were responsive to therapy with vitamin K antagonists, those who had recurrent thrombosis despite vitamin K antagonists, and those with a history of CAPS. Patients in the latter two groups (refractory and CAPS) had significantly higher levels of C5a and C5b-9 than those with a more favorable disease course (47). A different study evaluated a cohort of primary and secondary APS patients and found evidence of circulating immune complexes comprised of β_2GPI and aβ_2GPI antibodies (B2-CIC) in 39.3% of patients with APS. It was also shown that in patients with thrombotic APS, evidence of these circulating immune complexes was associated with thrombocytopenia, heart valve thickening/dysfunction, and lower levels of C3, and C4 (48). A recent 2022 study evaluated the significance of antibodies directed against factor Xa and thrombin (49). Anti-factor Xa antibodies (aFXa) and anti-thrombin antibodies (aThr) were affinity purified from patients with SLE ± APS (49). Interestingly, aThr potentiated thrombin-mediated activation of C3 and C5, while aFXa IgG did not increase C3 or C5 activation (49). Having said that, the longitudinal evaluation of 58 patients with SLE ± APS did not reveal a significant association between positivity for aFXa or aThr and C3 levels or disease activity (49); one wonders if a study of primary APS patients might show something different given the many factors that likely contribute to complement activation in SLE. A 2021 multicenter retrospective study evaluated preconception complement levels (C3 and C4) in 197 pregnant patients with APS or aPL and found that low preconception C3 and C4 were associated with a significantly higher prevalence of pregnancy losses (50). Furthermore, a subgroup analysis determined that patients with triple-positive aPL profiles and low complement levels were especially at risk (50). There have also been several case reports describing the successful use of eculizumab (an anti-C5 monoclonal antibody) in refractory cases of CAPS, as well as a recent analysis of eculizumab use amongst patients in the CAPS Registry (51–55).

Some exciting and/or recent insights regarding complement activation in APS are highlighted in Table 2. The recognition of the complement system as a critical mediator of APS pathogenesis is one of the most exciting discoveries in this field of research. Going forward, we look forward to seeing the extent to which detection of smoldering complement activation in seemingly stable patients will build momentum for immunomodulatory

approaches to treatment in a subset of individuals living with APS. It seems that additional work focused on the key upstream factors that lead to aberrant complement activation in APS is also likely to shine light on novel and more targeted approaches to therapy.

NEUTROPHILS AND NEUTROPHIL EXTRACELLULAR TRAPS

Neutrophils are the most abundant circulating leukocytes within the human body. They are a vital component of the innate immune system and are the first cells recruited to the site of infection or inflammation. Neutrophils are attracted to these injury sites by various chemotactic factors and their interactions with the endothelium and endothelial surface adhesion molecules (56). Once neutrophils are within the tissue, they can utilize a wide range of receptors to recognize microorganisms for phagocytosis (57). In addition to phagocytosis, neutrophils may fend off pathogens by generating reactive oxygen species, by delivering antimicrobial effectors into the extracellular space via degranulation, or by releasing extracellular meshworks of chromatin decorated with antimicrobial proteins known as neutrophil extracellular traps or NETs (58). NETs are released in response to diverse stimuli, including pathogens (bacteria, fungi, viruses, protozoa), activated platelets, activated endothelial cells, immune complexes, complement proteins, autoantibodies, and cytokines, amongst other factors (45). In the 1990s (prior to the first descriptions of NETs), it was shown that aβ_2GPI can activate neutrophils to release intracellular granules and produce hydrogen peroxide (59). In subsequent years, interest in the role of neutrophils and NETs in APS intensified. In the early 2000s, a study showed that neutrophils and C5a were essential mediators of fetal injury in a pregnancy model of APS; this work suggested a model whereby aPL triggered the formation of C5a on the surface of trophoblasts, which in turn caused the activation of neutrophils (60). Another mechanism by which neutrophils may become activated is via signaling mediated by tissue factor, factor VII, and protease-activated receptor 2 (40). A study from 2022 utilized APS as a model for arterial and venous thrombosis and showed that neutrophils were a driver of immunothrombosis (61). Deletion of kruppel-like factor 2 worsened disease in mice by driving neutrophil activation, whereas targeting P-selectin glycoprotein ligand 1 (PSGL-1) on activated neutrophils with immunoregulatory nanoparticles prevented downstream adhesion and thrombosis development (61).

NETs have been implicated in causing thrombosis through multiple mechanisms, including activation of coagulation factors, the endothelium, platelets, and the complement cascade (62). A 2014 study found that sera of APS patients had a decreased ability to degrade NETs, which is potentially associated with increased levels of antibodies directed against NETs and neutrophil remnants (63). More recent research has formalized the concept of anti-NET antibodies as players in APS pathogenesis. One study found significantly elevated levels of anti-NET antibodies (both IgG and IgM) in patients with primary APS as compared with healthy controls, where they impaired degradation of NETs (especially the IgG isotype) and inversely correlated with complement C4 (IgM isotype) (64). These findings suggest that anti-NET antibodies promote inflammation by hindering the clearance of proinflammatory NETs, thereby amplifying complement activation.

In 2014, Yalavarthi and colleagues first demonstrated a direct role of NETs in APS pathogenesis (65). Sera and plasma from primary APS patients had elevated levels of NETs as compared with healthy volunteers, while APS patient neutrophils appeared primed for spontaneous NET release as compared with control neutrophils. The authors also found that aPL-mediated release of NETs required reactive oxygen species and TLR4 signaling (65), which has been replicated by other groups (66). A recent study evaluating risk factors associated with thrombosis in APS found a positive correlation between the level of NETs and activated protein C (APC) resistance (67). This correlation was particularly robust in the setting of high-risk aPL profiles (e.g., triple-positive aPL) (67). Another recent study also supports the association between thrombotic APS and increased NET formation (68). By evaluating the expression of pro-NETosis genes (*PADI4*, *ELANE*, and *MPO*), as well as circulating NET remnants (citrullinated histone H3 and myeloperoxidase-DNA complexes), the authors found a pronounced association between thrombotic APS (particularly those patients with triple aPL positivity or recurrent thrombosis) and increased NET formation (68). An additional 2022 study also found that leukocyte immunoglobulin receptor A3 (LILRA3) was significantly increased in patients with thrombotic APS (69). Furthermore, it was shown that LILRA3 was positively correlated with myeloperoxidase-DNA complexes (NET remnants) in patients with LILRA3-positive thrombotic APS (69). After treatment, the levels of LILRA3 and myeloperoxidase-DNA complexes were consistently decreased amongst these patients, suggesting that LILRA3 might eventually be leveraged as a biomarker or therapeutic target in thrombotic APS (69).

Several strategies for inhibiting APS-associated NET release have been studied in preclinical models. Successful approaches have included neutrophil depletion, deoxyribonuclease administration (dissolves NETs), activation of neutrophil surface adenosine receptors, and administration of phosphodiesterase inhibitors (70–73). A recent study put forth the idea that the polyanionic compound defibrotide (first reported as a potential therapy for CAPS some 20 years ago (74)) has a protective role in part through inhibition of NET release (75).

Some notable and/or recent insights regarding neutrophil activation in APS are highlighted in Table 3. If we assume that hyperactive neutrophils are essential players in the exaggerated innate immune responses that propel thrombotic events, and potentially other manifestations, in APS, then creative approaches, including manipulation of neutrophil metabolism, may be necessary to bring viable therapeutic strategies to the clinic. Since neutrophils are well established to interact with both endothelial cells and platelets, a complete understanding of APS pathophysiology will likely require careful dissection of intercellular communication in APS.

CONCLUSION

In summary, we know that APS is an acquired thrombo-inflammatory disease with diverse criteria and non-criteria clinical manifestations. In recent years we have gained more understanding of the various mechanisms by which APS manifestations occur. Several years of APS research have shed light on the multiple pathways that lead to endothelial activation and injury, the involvement of complement in obstetric APS and activation of the coagulation cascade, and on neutrophil activation and the formation of NETs. More

recent work in APS has revealed the importance of extracellular vesicles, circulating endothelial cells, circulating immune complexes, complex interactions between complement and anticoagulation, and anti-NET antibodies. Although our understanding of the underlying pathophysiology of APS has improved significantly, many aspects of the disease process and autoimmunity can be explored further. As we delve further into the pathogenesis of APS, we should consider a multi-omics approach in our evaluation of patient cohorts. This type of analysis allows for more detailed stratification of patients by phenotypic profiles, not limited by clinical data alone. A study from 2021 performed unsupervised clustering of whole blood transcriptome data of patients with systemic autoimmune disease (including a few with APS) (76). The transcriptome data revealed three aberrant clusters: inflammatory, lymphoid, and interferon patterns. Furthermore, this study interrogated an independent inception cohort and found that patients stratified to the same pathological clusters over time (14 months) (76). As research in APS moves forward, the availability of transcriptome data will allow for increased granularity in the stratification of patient phenotypes, which will undoubtedly help identify novel aspects of pathogenesis and aid in the development of safer and more effective therapies for patients.

FINANCIAL SUPPORT AND SPONSORSHIP

YZ was supported by grants from the Rheumatology Research Foundation, the Arthritis National Research Foundation, and the NIH (K08 AR080205). JSK was supported by grants from the Lupus Research Alliance, Rheumatology Research Foundation, and NIH (R01 HL134846).

REFERENCES

1. Cohen H, Cuadrado MJ, Erkan D, et al. 16th International Congress on Antiphospholipid Antibodies Task Force Report on Antiphospholipid Syndrome Treatment Trends. Lupus. 2020;29(12):1571–93. [PubMed: 33100166]
2. Tektonidou MG, Andreoli L, Limper M, et al. EULAR recommendations for the management of antiphospholipid syndrome in adults. Ann Rheum Dis. 2019;78(10):1296–304. [PubMed: 31092409]
3. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4(2):295–306. [PubMed: 16420554]
4. Pignatelli P, Ettorre E, Menichelli D, et al. Seronegative antiphospholipid syndrome: refining the value of “non-criteria” antibodies for diagnosis and clinical management. Haematologica. 2020;105(3):562–72. [PubMed: 32001534]
5. Duarte-Garcia A, Pham MM, Crowson CS, et al. The Epidemiology of Antiphospholipid Syndrome: A Population-Based Study. Arthritis Rheumatol. 2019;71(9):1545–52. [PubMed: 30957430]
6. Cervera R, Khamashta MA, Shoenfeld Y, et al. Morbidity and mortality in the antiphospholipid syndrome during a 5-year period: a multicentre prospective study of 1000 patients. Ann Rheum Dis. 2009;68(9):1428–32. [PubMed: 18801761]
7. Cervera R, Piette JC, Font J, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. Arthritis Rheum. 2002;46(4):1019–27. [PubMed: 11953980]
8. Erkan D, Espinosa G, Cervera R. Catastrophic antiphospholipid syndrome: updated diagnostic algorithms. Autoimmun Rev. 2010;10(2):74–9. [PubMed: 20696282]
9. 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):2097–110. [PubMed: 29500169]

10. Ramesh S, Morrell CN, Tarango C, et al. Antiphospholipid antibodies promote leukocyte-endothelial cell adhesion and thrombosis in mice by antagonizing eNOS via beta2GPI and apoER2. J Clin Invest. 2011;121(1):120–31. [PubMed: 21123944]
11. Allen KL, Fonseca FV, Betapudi V, et al. A novel pathway for human endothelial cell activation by antiphospholipid/anti-beta2 glycoprotein I antibodies. Blood. 2012;119(3):884–93. [PubMed: 22106343]
12. Ma K, Simantov R, Zhang JC, et al. High affinity binding of beta 2-glycoprotein I to human endothelial cells is mediated by annexin II. J Biol Chem. 2000;275(20):15541–8. [PubMed: 10809787]
13. Zhang J, McCrae KR. Annexin A2 mediates endothelial cell activation by antiphospholipid/anti-beta2 glycoprotein I antibodies. Blood. 2005;105(5):1964–9. [PubMed: 15471954]
14. 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):3074–83. [PubMed: 19628708]
15. Allen KL, Hamik A, Jain MK, McCrae KR. Endothelial cell activation by antiphospholipid antibodies is modulated by Kruppel-like transcription factors. Blood. 2011;117(23):6383–91. [PubMed: 21482710]
16. Vega-Ostertag M, Casper K, Swerlick R, et al. Involvement of p38 MAPK in the upregulation of tissue factor on endothelial cells by antiphospholipid antibodies. Arthritis Rheum. 2005;52(5):1545–54. [PubMed: 15880836]
17. Dunoyer-Geindre S, de Moerloose P, Galve-de Rochemonteix B, et al. NFkappaB is an essential intermediate in the activation of endothelial cells by anti-beta(2)-glycoprotein 1 antibodies. Thromb Haemost. 2002;88(5):851–7. [PubMed: 12428105]
18. Pierangeli SS, Espinola RG, Liu X, Harris EN. Thrombogenic effects of antiphospholipid antibodies are mediated by intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and P-selectin. Circ Res. 2001;88(2):245–50. [PubMed: 11157679]
19. Espinola RG, Liu X, Colden-Stanfield M, et al. E-Selectin mediates pathogenic effects of antiphospholipid antibodies. J Thromb Haemost. 2003;1(4):843–8. [PubMed: 12871424]
20. Nochy D, Daugas E, Droz D, et al. The intrarenal vascular lesions associated with primary antiphospholipid syndrome. J Am Soc Nephrol. 1999;10(3):507–18. [PubMed: 10073601]
21. Daugas E, Nochy D, Huong DLT, et al. Antiphospholipid syndrome nephropathy in systemic lupus erythematosus. J Am Soc Nephrol. 2002;13(1):42–52. [PubMed: 11752020]
22. Tektonidou MG, Sotsiou F, Nakopoulou L, et al. Antiphospholipid syndrome nephropathy in patients with systemic lupus erythematosus and antiphospholipid antibodies: prevalence, clinical associations, and long-term outcome. Arthritis Rheum. 2004;50(8):2569–79. [PubMed: 15334471]
23. Long BR, Leya F. The role of antiphospholipid syndrome in cardiovascular disease. Hematol Oncol Clin North Am. 2008;22(1):79–94, vi–vii. [PubMed: 18207067]
24. Alarcon-Segovia D, Cardiel MH, Reyes E. Antiphospholipid arterial vasculopathy. J Rheumatol. 1989;16(6):762–7. [PubMed: 2778758]
25. Hughson MD, McCarty GA, Brumback RA. Spectrum of vascular pathology affecting patients with the antiphospholipid syndrome. Hum Pathol. 1995;26(7):716–24. [PubMed: 7628842]
26. Manning BD, Toker A. AKT/PKB Signaling: Navigating the Network. Cell. 2017;169(3):381–405. [PubMed: 28431241]
27. Canaud G, Bienaime F, Tabarin F, et al. Inhibition of the mTORC pathway in the antiphospholipid syndrome. N Engl J Med. 2014;371(4):303–12. [PubMed: 25054716]
28. Sevim E, Siddique S, Chalasani MLS, et al. Mammalian Target of Rapamycin Pathway Assessment in Antiphospholipid Antibody-Positive Patients with Livedo. J Rheumatol. 2022.
29. Blann AD, Woywodt A, Bertolini F, et al. Circulating endothelial cells. Biomarker of vascular disease. Thromb Haemost. 2005;93(2):228–35. [PubMed: 15711737]
30. Martinez-Sales V, Vila V, Mico L, et al. [Circulating endothelial cells and microparticles in patients with antiphospholipid antibodies]. Med Clin (Barc). 2011;136(10):431–3. [PubMed: 21051055]
31. Foret T, Dufrost V, Heymonet M, et al. Circulating endothelial cells are associated with thromboembolic events in patients with antiphospholipid antibodies. Thromb Haemost. 2022.**This study evaluated levels of CECs in patients with aPL and/or SLE. The authors

found notable correlations between CECs and various clinical risk factors as well as markers of endothelial dysfunction

32. Tan Y, Bian Y, Song Y, et al. Exosome-Contained APOH Associated With Antiphospholipid Syndrome. Front Immunol. 2021;12:604222.*This work provides insights on the association between circulating exosomes in pregnant APS patients and fetal complications
33. Velasquez M, Pelaez LF, Rojas M, et al. Differences in Endothelial Activation and Dysfunction Induced by Antiphospholipid Antibodies Among Groups of Patients With Thrombotic, Refractory, and Non-refractory Antiphospholipid Syndrome. Front Physiol. 2021;12:764702.**This study correlated distinct clinical APS phenotypes with differential expression of markers related to endothelial activation and dysfunction
34. Rodriguez CM, Velasquez-Berrio M, Rua C, et al. Antiphospholipid Antibodies From Women With Pregnancy Morbidity and Vascular Thrombosis Induce Endothelial Mitochondrial Dysfunction, mTOR Activation, and Autophagy. Front Physiol. 2021;12:706743.
35. Zhang G, He C, Wu Q, et al. Impaired Autophagy Induced by oxLDL/beta2GPI/anti-beta2GPI Complex through PI3K/AKT/mTOR and eNOS Signaling Pathways Contributes to Endothelial Cell Dysfunction. Oxid Med Cell Longev. 2021;2021:6662225.
36. Dong Y, Lu Y, Xia Y, Wang X. Effect of hydroxychloroquine on antiphospholipid antibodies-inhibited endometrial angiogenesis. J Matern Fetal Neonatal Med. 2021:1–9.
37. Capozzi A, Riitano G, Recalchi S, et al. Effect of heparanase inhibitor on tissue factor overexpression in platelets and endothelial cells induced by anti-beta2-GPI antibodies. J Thromb Haemost. 2021;19(9):2302–13. [PubMed: 34107171]
38. Shi H, Zuo Y, Navaz S, et al. Endothelial Cell-Activating Antibodies in COVID-19. Arthritis Rheumatol. 2022;74(7):1132–8. [PubMed: 35174669]
39. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement System Part I - Molecular Mechanisms of Activation and Regulation. Front Immunol. 2015;6:262. [PubMed: 26082779]
40. 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(7):2423–31. [PubMed: 17536017]
41. Salmon JE, Girardi G. Antiphospholipid antibodies and pregnancy loss: a disorder of inflammation. J Reprod Immunol. 2008;77(1):51–6. [PubMed: 17418423]
42. Pierangeli SS, Girardi G, Vega-Ostertag M, et al. Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia. Arthritis Rheum. 2005;52(7):2120–4. [PubMed: 15986360]
43. Romay-Penabad Z, Liu XX, Montiel-Manzano G, et al. C5a receptor-deficient mice are protected from thrombophilia and endothelial cell activation induced by some antiphospholipid antibodies. Ann N Y Acad Sci. 2007;1108:554–66. [PubMed: 17894020]
44. Carrera-Marin A, Romay-Penabad Z, Papalardo E, et al. C6 knock-out mice are protected from thrombophilia mediated by antiphospholipid antibodies. Lupus. 2012;21(14):1497–505. [PubMed: 22933620]
45. Brinkmann V. Neutrophil Extracellular Traps in the Second Decade. J Innate Immun. 2018;10(5–6):414–21. [PubMed: 29909412]
46. Grosso G, Sandholm K, Antovic A, et al. The Complex Relationship between C4b-Binding Protein, Warfarin, and Antiphospholipid Antibodies. Thromb Haemost. 2021;121(10):1299–309. [PubMed: 33412597] *The authors found that an inhibitor of the classical and lectin complement pathways (C4BPt) was significantly reduced in persistently aPL-positive patients
47. Ruffatti A, Tonello M, Calligaro A, et al. High plasma C5a and C5b-9 levels during quiescent phases are associated to severe antiphospholipid syndrome subsets. Clin Exp Rheumatol. 2022.**This study showed that APS patients with a history of non-favorable disease course had elevated complement activation products (C5a and C5b-9) despite having quiescent disease.
48. Naranjo L, Stojanovich L, Djokovic A, et al. Circulating immune-complexes of IgG/IgM bound to B2-glycoprotein-I associated with complement consumption and thrombocytopenia in antiphospholipid syndrome. Front Immunol. 2022;13:957201.**The authors evaluated circulating

immune complexes of β2GPI and aβ2GPI antibodies in APS patients, and found that they were associated with thrombocytopenia, heart valve thickening/dysfunction, and lower C3 and C4 levels

49. McDonnell T, Amarnani R, Spicer C, et al. Antibodies to FXa and thrombin in patients with SLE differentially regulate C3 and C5 cleavage. Lupus Sci Med. 2022;9(1).
50. Nalli C, Lini D, Andreoli L, et al. Low Preconception Complement Levels Are Associated with Adverse Pregnancy Outcomes in a Multicenter Study of 260 Pregnancies in 197 Women with Antiphospholipid Syndrome or Carriers of Antiphospholipid Antibodies. Biomedicines. 2021;9(6).*This study evaluated preconception complement levels in pregnant patients with either APS or aPL and showed that those with low preconception C3 and C4 had higher prevalence of pregnancy loss, particularly in the setting of high-risk aPL profiles.
51. Lopez-Benjume B, Rodriguez-Pinto I, Amigo MC, et al. Eculizumab use in catastrophic antiphospholipid syndrome (CAPS): Descriptive analysis from the “CAPS Registry”. Autoimmun Rev. 2022;21(4):103055.
52. Hussain H, Tarantino MD, Chaturvedi S, et al. Eculizumab for refractory thrombosis in antiphospholipid syndrome. Blood Adv. 2022;6(4):1271–7. [PubMed: 35051999]
53. Faguer S, Ribes D. Early use of eculizumab for catastrophic antiphospholipid syndrome. Br J Haematol. 2022;196(2):e12–e4. [PubMed: 34396515]
54. Chidharla A, Syed SB, Chatterjee T, Tarantino MD. A Case Report of COVID-Associated Catastrophic Antiphospholipid Syndrome Successfully Treated with Eculizumab. J Blood Med. 2021;12:929–33. [PubMed: 34744467]
55. Nauseef JT, Lim HI, DeSancho MT. Successful outcome with eculizumab treatment in a patient with antiphospholipid syndrome presenting with an unusual thrombotic storm. J Thromb Thrombolysis. 2021;52(2):597–600. [PubMed: 33222114]
56. Ng LG, Ostuni R, Hidalgo A. Heterogeneity of neutrophils. Nat Rev Immunol. 2019;19(4):255–65. [PubMed: 30816340]
57. Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. Annu Rev Pathol. 2014;9:181–218. [PubMed: 24050624]
58. Kaplan MJ, Radic M. Neutrophil extracellular traps: double-edged swords of innate immunity. J Immunol. 2012;189(6):2689–95. [PubMed: 22956760]
59. Arvieux J, Jacob MC, Roussel B, et al. Neutrophil activation by anti-beta 2 glycoprotein I monoclonal antibodies via Fc gamma receptor II. J Leukoc Biol. 1995;57(3):387–94. [PubMed: 7884309]
60. 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):1644–54. [PubMed: 14660741]
61. Nayak L, Sweet DR, Thomas A, et al. A targetable pathway in neutrophils mitigates both arterial and venous thrombosis. Sci Transl Med. 2022;14(660):eabj7465.**This study confirms the critical role of neutrophils in models of APS-associated arterial and venous thrombosis while suggesting that diminished activity of the transcription factor KLF2 unlocks neutrophil effector functions in APS.
62. Laridan E, Martinod K, De Meyer SF. Neutrophil Extracellular Traps in Arterial and Venous Thrombosis. Semin Thromb Hemost. 2019;45(1):86–93. [PubMed: 30634198]
63. Leffler J, Stojanovich L, Shoenfeld Y, et al. Degradation of neutrophil extracellular traps is decreased in patients with antiphospholipid syndrome. Clin Exp Rheumatol. 2014;32(1):66–70. [PubMed: 24295292]
64. Zuo Y, Yalavarthi S, Gockman K, et al. Anti-Neutrophil Extracellular Trap Antibodies and Impaired Neutrophil Extracellular Trap Degradation in Antiphospholipid Syndrome. Arthritis Rheumatol. 2020;72(12):2130–5. [PubMed: 32729667]
65. Yalavarthi S, Gould TJ, Rao AN, et al. Release of neutrophil extracellular traps by neutrophils stimulated with antiphospholipid antibodies: a newly identified mechanism of thrombosis in the antiphospholipid syndrome. Arthritis Rheumatol. 2015;67(11):2990–3003. [PubMed: 26097119]
66. Zha C, Zhang W, Gao F, et al. Anti-beta2GPI/beta2GPI induces neutrophil extracellular traps formation to promote thrombogenesis via the TLR4/MyD88/MAPKs axis activation. Neuropharmacology. 2018;138:140–50. [PubMed: 29883691]

67. Foret T, Dufrost V, Salomon du Mont L, et al. A new pro-thrombotic mechanism of neutrophil extracellular traps in antiphospholipid syndrome: impact on activated protein C resistance. Rheumatology (Oxford). 2022;61(7):2993–8. [PubMed: 34791113]

68. Mazetto BM, Hounkpe BW, da Silva Saraiva S, et al. Association between neutrophil extracellular traps (NETs) and thrombosis in antiphospholipid syndrome. Thromb Res. 2022;214:132–7. [PubMed: 35561448] *This work further supports the association between thrombotic APS and increased NET formation through the evaluation of expression of pro-NETosis genes and circulating NET remnants.

69. Liu H, Li C, Shi H, et al. Soluble LILRA3 is aberrantly expressed in antiphospholipid syndrome (APS) and is a potential marker of thrombotic APS. Rheumatology (Oxford). 2022.**This study found that leukocyte immunoglobulin receptor A3 (LILRA3) was significantly elevated in patients with thrombotic APS. This study also suggested that LILRA3 could potentially be leveraged as a biomarker or therapeutic target due to its consistent decrease in patients after treatment.

70. Ali RA, Gandhi AA, Meng H, et al. Adenosine receptor agonism protects against NETosis and thrombosis in antiphospholipid syndrome. Nat Commun. 2019;10(1):1916. [PubMed: 31015489]

71. Ali RA, Estes SK, Gandhi AA, et al. Defibrotide inhibits antiphospholipid antibody-mediated NET formation and venous thrombosis. Arthritis Rheumatol. 2021.

72. Ali RA, Gandhi AA, Dai L, et al. Antineutrophil properties of natural gingerols in models of lupus. JCI Insight. 2021;6(3).

73. Meng H, Yalavarthi S, Kanthi Y, et al. In Vivo Role of Neutrophil Extracellular Traps in Antiphospholipid Antibody-Mediated Venous Thrombosis. Arthritis Rheumatol. 2017;69(3):655–67. [PubMed: 27696751]

74. Burcoglu-O'Ral A, Erkan D, Asherson R. Treatment of catastrophic antiphospholipid syndrome with defibrotide, a proposed vascular endothelial cell modulator. J Rheumatol. 2002;29(9):2006–11. [PubMed: 12233899]

75. Ali RA, Estes SK, Gandhi AA, et al. Defibrotide Inhibits Antiphospholipid Antibody-Mediated Neutrophil Extracellular Trap Formation and Venous Thrombosis. Arthritis Rheumatol. 2022;74(5):902–7. [PubMed: 34725956] *Following up on a decades-old case report, this study provides mechanistic insights into how defibrotide restrains APS-mediated thrombo-inflammation

76. Barturen G, Babaei S, Catala-Moll F, et al. Integrative Analysis Reveals a Molecular Stratification of Systemic Autoimmune Diseases. Arthritis Rheumatol. 2021;73(6):1073–85. [PubMed: 33497037] **This interesting study utilized whole blood transcriptome analysis of patients with autoimmune disease (including those with APS) and found three distinct clusters that were stable over time.

KEY POINTS

- Endothelial cells can be activated by antiphospholipid antibodies, and there is significant opportunity to understand the relative roles of different antibody specificities in this process (for example, aβ_2GPI versus anti-prothrombin).
- The detection of smoldering complement activation in many patients with APS emphasizes that a subset of patients would likely benefit from some type of immunomodulatory therapy.
- Neutrophil extracellular traps (NETs) are likely important end effectors of thrombotic events in APS, but how these might be neutralized in patients remains elusive.
- The identification of more nuanced approaches to phenotyping patients will hopefully pave the way for developing safer and more effective patient-specific therapeutic strategies for APS.

Table 1:

Insights regarding endothelial activation and Injury

Pathway	Summary & Effects	References	Key Study Details
ApoER2	Mediates aPL-induced activation of protein phosphatase 2A (PP2A), which leads to antagonism of eNOS and promotion of thrombosis.	Sacharidoi et al., 2018 (9)	-Wild-type mice and mice lacking endothelial apoER2
Annexin A2	Mediates binding of aPL and β_2GPI to endothelial cells and, when deleted, protects mice from aPL-mediated thrombosis.	Ma et al., 2000 (12) Zhang et al., 2005 (13) Romay-Penabad et al., 2009 (14)	-In vitro, HUVECs -In vitro, HUVECs -Thrombosis model with annexin A2-deficient mice
TLR4	Plays a critical role in anti-β_2GPI-mediated endothelial cell activation through the assembly of a multi-protein signaling complex on the endothelial cell surface.	Allen et al., 2012 (11)	-In vitro, HUVECs
P38 MAPK, NF-kB	Mediate an increased production of IL-6 and IL-8, and heightened tissue factor activity in HUVECs after exposure to aPL.	Vega-Ostertag et al., 2005 (16)	-In vitro, HUVECs
eNOS	Antagonism of eNOS by aPL promotes leukocyte adhesion to endothelial cells.	Ramesh et al., 2011 (10)	-In vitro, HAECs and BAECs -ApoER2 and eNOS positive and deficient mice
Kruppel-like transcription factors	Endothelial Kruppel-like factors 2 and 4 expression are diminished by aPL, which negatively impacts vascular homeostasis.	Allen et al., 2011 (15)	-In vitro, HUVECs
mTOR	Renal biopsies in APS-associated nephropathy show activation of mTOR pathway. Skin biopsies of aPL-positive patients with livedo reticularis/racemosa had evidence of increased mTOR activity.	Canaud et al., 2014 (27) Sevim et al., 2022 (28)	-In vitro, immunostaining of biopsies
Circulating endothelial cells (CECs)	CECs are elevated in APS where they correlate with markers of endothelial dysfunction.	Blann et al., 2005 (29) Foret et al., 2022 (31)	-In vitro, patient blood
Extracellular vesicles or exosomes	Exosomes isolated from APS patients were associated with increased fetal resorption in a murine pregnancy model.	Tan et al., 2021 (32)	-In vitro, patient blood, murine pregnancy model

Table 2:

Complement Activation and Complement Mediated Injury

Pathway	Summary & Effects	References	Key Study Details
C5a	C5a interaction with C5a receptor can lead to endothelial activation and increased release of tissue factor. C5a deposition can lead to neutrophil activation.	Redecha et al., 2007 (40) Girardi et al., 2003 (60)	-C5aR and C3aR deficient mice -Murine pregnancy model
C3, C5, C6	Deficiencies in complement components such as C3, C5, and C6 are protective from thrombophilia and endothelial activation induced by aPL.	Pierangeli et al., 2005 (42) Romay-Penabad et al., 2007 (43) Carrera-Marin et al., 2012 (44)	-Mice deficient in various complement components.
C4b-binding protein (C4BPt; inhibitor of the classical and lectin complement cascades)	C4BPt is negatively associated with complement activation products, and a sizable percentage of the reduction in C4BPt in aPL-positive patients was attributed to warfarin administration.	Grosso et al., 2021 (46)	-In vitro, patient blood
Anti-thrombin antibodies (aThr)	In patients with SLE ± APS, aTHr potentiates thrombin-mediated activation of C3 and C5.	McDonnell et al., 2022 (49)	-In vitro, patient blood
C3 and C4 (preconception levels)	Low preconception levels of C3 and C4 in APS or aPL patients are associated with a significantly higher prevalence of pregnancy loss.	Nalli et al., 2021 (50)	-In vitro, patient blood

Table 3:

Neutrophils and Neutrophil Extracellular Traps (NETs)

Pathway	Summary & Effects	References	Key Study Details
NETs clearance	APS patient serum is associated with a decreased ability to degrade NETs.	Leffler et al., 2014 (63)	-In vitro, patient blood
TLR4-dependent aPL-mediated NETosis	The aβ_2GPI/β_2GPI immune complex induces NETs formation in a time and concentration dependent manner, which is mediated by the TLR4 pathway.	Zha et al., 2018 (66)	-In vitro, patient blood -carotid artery thrombosis mouse model
Activated protein C resistance	The level of NETs in APS patients positively correlates with activated protein C resistance. This correlation is more robust in triple-positive patients.	Foret et al., 2022 (67)	-In vitro, patient blood
Anti-NET antibodies	Many APS patients have a significant elevation of anti-NET antibodies, which impair the degradation of NETs and activate complement.	Zuo et al., 2020 (64)	-In vitro, patient blood
Pro-NETosis genes (PADI4, ELANE, MPO) and NET remnants	There is a significant association between thrombotic APS and increased NET formation, as evidenced by increased expression of pro-NETosis genes and NET remnants.	Mazetto et al., 2022 (68)	-In vitro, patient blood
LILRA3 (leukocyte immunoglobulin receptor A3)	LILRA3 is significantly increased in patients with thrombotic APS. Levels of LILRA3 and NET remnants were decreased after treatment.	Liu et al., 2022 (69)	-In vitro, patient blood