Purpose of review  
To review novel therapeutic targets that are currently under investigation to develop safer, targeted therapies for antiphospholipid antibody (aPL)-mediated clinical manifestations.

Recent findings  
Novel therapeutic options potentially available include anti-CD20 monoclonal antibodies and new-generation anticoagulants (such as direct thrombin and anti-Xa inhibitors). Research focusing on interfering with aPL-mediated cell activation, targeting complement components and the innovative concept of blocking the pathogenic subpopulation of aPL with tailored peptides are currently being explored.

Summary  
Antiphospholipid syndrome is an autoimmune disease characterized by thrombosis and pregnancy morbidity occurring in patients persistently positive for aPL. Current therapeutic options remain confined to long-term anticoagulation with vitamin K antagonists. The future holds much promise with the identification of novel potential targets, many of which are currently under investigation. The challenge will be to design prospective randomized controlled clinical trials to provide the evidence necessary to support integration of these therapies into clinical practice.

Keywords  
antiphospholipid antibodies, antiphospholipid syndrome, novel therapy, pregnancy loss, thrombosis

INTRODUCTION

The antiphospholipid syndrome (APS) is an autoimmune systemic disease characterized by vascular thrombosis and/or pregnancy morbidity occurring in patients persistently tested positive for antiphospholipid antibodies (aPL) [1]. Currently, the only proven therapeutic option is long-term anticoagulation with vitamin K antagonists [2]. Indeed, management of APS centres on attenuating the procoagulant state while balancing the risks of anticoagulant therapy. However, despite the extensive use of long-term anticoagulation in the prevention and management of APS, there are ongoing concerns about efficacy and safety, including the narrow therapeutic window and numerous drug and dietary interactions. Adherence to treatment and the impact of long-term anticoagulation on daily life present many challenges for patients with APS, not least of which include so-called ‘noncriteria’ manifestations such as cognitive dysfunction and fatigue [3]. Dietary interactions and regular, often frequent monitoring of the international normalized ratio (INR), which is inconvenient and costly, but essential to maintain the INR within the target therapeutic range are the most frequently reported patient concerns. Moreover, monitoring anticoagulation with vitamin K antagonists in patients with APS can be a real challenge, as the responsiveness of reagents used in the INR test in lupus anticoagulant positive patients varies widely, potentially leading to the instability of anticoagulation [4]. Possibly because of these difficulties, recurrent thromboses and obstetric complications occur despite apparently optimum therapy. There is no consensus among experts on the best management or alternative options in such difficult cases.

There is therefore a major unmet need for novel therapies that ideally can avoid the need for regular INR testing but at the same time provide assurance that APS patients will be protected from further events. The use of novel anticoagulants in APS patients with venous thrombosis is being evaluated...
and until the trial data are available, patients remain on standard vitamin K antagonists such as warfarin. Recent progress in understanding the pathogenic mechanisms of APS has opened new horizons for targeted therapies. Anti-inflammatory and immunomodulatory pathways are currently being explored and represent promising approaches [5,6] (Fig. 1).

In this manuscript, we review novel therapeutic targets that are under investigation for the treatment of APS.

NEW ORAL ANTICOAGULANTS

The new oral anticoagulants include dabigatran etexilate (Pradaxa, Boehringer Ingelheim Pharma GmbH & Co), a direct thrombin inhibitor, rivaroxaban (Xarelto, Bayer Pharma), apixaban (Eliquis, Bristol-Myers Squibb Pharmaceuticals) and edoxaban (Lixiana, Daiichi Sankyo Company), which are direct anti-Xa inhibitors. These emerging agents represent a major advance as, unlike the vitamin K antagonists, they have few reported drug interactions that could affect anticoagulant intensity and

**FIGURE 1.** Proposed target approaches for APS. (1) Depletion of B cells by anti CD20 monoclonal antibodies, to reduce pathogenic anti-phospholipid antibody (aPL); (2) use of small inhibitory peptides modeled on domain V (TFI) or domain I of β2GPI; (3) blocking of aPL/β2GPI receptors on target cells (annexin A2 and Toll-like receptor 4 on endothelial cell and monocytes; lipoprotein receptor-related protein family on platelets; (4) inhibitors of complement components; (5) inhibitors of intracellular pathway (p38MAPK and NF-kB), including BIRB796 BS, SB203580, VX702; (6) reducing arachidonic acid production inhibiting cytosolic phospholipases A2 is one of the proposed antithrombotic mechanisms of hydroxychloroquine; (7) tissue factor inhibitors, including ACE inhibitors, pentoxifylline, an adenosine uptake inhibitor (dilazep), and ss-deoxyribonucleic acid derivatives (e.g. defibrotide) and newly developed antitissue factor monoclonal antibodies; (8) new generation anticoagulants targeting FXa or thrombin. AA, arachidonic acid; cPLA2, cytosolic phospholipases A2.
they seem not to interact with dietary foods [4,7,8]. All these agents have very predictable anticoagulant effects with fixed dosing regimens, making it unnecessary to routinely monitor anticoagulant intensity. Although this is very appealing from a patient perspective, one of the advantages of traditional vitamin K antagonists is that measuring and recording the INR ensures adherence to therapy. The lack of INR monitoring with the novel agents may introduce variability in patient adherence to therapy which could be hard to detect, an issue which is generally under recognized with any oral therapy. APS patients are unique in that, anecdotally, many patients have a sense of their INR and feel unwell when it is below the target range, something that does not occur in other thrombotic disorders. It remains to be seen whether patients report similar symptoms with these novel agents.

The efficacy of the novel anticoagulants has been demonstrated in large phase III clinical trials [9,10], and both rivaroxaban and dabigatran have been licensed by the European Medicines Agency for the prevention of stroke and systemic embolism in patients with atrial fibrillation [11,12]. In a randomized, double-blind, noninferiority trial involving patients with acute venous thromboembolism, a fixed dose of oral dabigatran was found as effective and well tolerated as warfarin, with the advantage of not requiring laboratory monitoring [13]. Rivaroxaban was superior to vitamin K antagonists in preventing a recurrent thrombotic event following 12 months’ postprimary event warfarin administration [14]. These studies demonstrated low incidences of major bleeding, with dabigatran being at least similar if not superior to warfarin. These new agents would be expected to improve the quality of life in APS patients. However, in order to assess whether these new agents are as effective as warfarin in APS patients, we are currently conducting the Rivaroxaban in Antiphospholipid Syndrome (RAPS, IRSCTN 68222801) trial. RAPS is a prospective randomized controlled trial of warfarin versus rivaroxaban in patients with venous thrombotic APS, with or without systemic lupus erythematosus (SLE), being maintained at a target INR of 2.5 (i.e. range 2.0–3.0) [15].

**HYDROXYCHLOROQUINE**

Hydroxychloroquine (HCQ) has been proposed to reduce the risk of thrombosis in clinical studies [16,17] and animal models of APS. Its potential antithrombotic mechanisms include inhibition of platelet aggregation and adhesion [18], cholesterol-lowering mechanisms [18] and blockade of aPL production [19]. Rand et al. [20] showed that HCQ significantly reduced the binding of aPL-β2GPI complexes to phospholipid surfaces. The drug also reduced the binding of the individual proteins to bilayers. The same study [21] observed that HCQ also caused modest, but statistically significant, reductions of clinical antiphospholipid antibody titers. HCQ was also found to reduce the disruption by aPL of the annexin A5 anticoagulant shield. A beneficial effect of HCQ on primary thrombosis prevention in aPL-positive patients was shown in both retrospective and prospective studies [22,23]. Kaiser et al. [23] confirmed, in a large and ethnically diverse SLE cohort including 1930 patients, that HCQ use was protective for thrombosis.

Recently, Albert et al. [24] showed that HCQ reversed the aPL-inhibition of trophoblast interleukin (IL)-6 secretion and partially limited aPL-inhibition of cell migration, suggesting that some form of combination therapy that includes HCQ may be beneficial to pregnant APS patients. A randomized multicenter study [25] has been initiated to address the prophylactic role of HCQ against thrombosis in patients with aPL.

**STATINS**

Statins are a class of drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase. They have been shown to have additional anti-inflammatory, immunoregulatory and antithrombotic effects *in vitro* and *in vivo* [26,27]. Their efficacy in primary and secondary prevention of cardiovascular events [28,29] and venous thrombosis [30] has been demonstrated in the general population. Statins have been shown to decrease aPL-induced endothelial cell activation via inhibition of the expression of adhesion molecules and IL-6 and by reversal of tissue factor (TF) upregulation [31–34]. Statins also have a variety of direct effects on gene expression and on the function of cells of both the innate and adaptive immune systems [35]. By altering isoprenylation, which in turn induces the inhibition of the small GTP-binding proteins Rho, Ras and Rac, statins are able to decrease oxidative stress and inflammation, inhibit the thrombogenic response, and exert beneficial effects on the immune system [36]. In APS, statins have been demonstrated to interfere with monocyte, lymphocyte and endothelial cell function, all of which may contribute to thrombosis prevention in aPL positive patients [32,37,38]. Preliminary results of an ongoing phase II clinical trial investigating the levels of proinflammatory/prothrombotic biomarkers in aPL patients reported a significant decrease in vascular endothelial growth factor, soluble TF and TNFα titres after 30 days’
therapy with fluvastatin (40 mg/daily) [39,40]. Randomized controlled trials are needed to confirm these promising preliminary results supporting the effectiveness of statins in the prevention of thrombosis in aPL-positive patients. The major difficulty with primary prevention trials of this nature is the very large sample size needed to provide a definitive answer, and this is challenging in a condition like APS. In-vitro evidence for the use of pravastatin in pregnancy is still not conclusive, and its role in preventing pregnancy complications in patients positive for aPL is still debated [41]. The in-vivo condition may be more complex, and the effectiveness of pravastatin in the prevention of aPL-associated pregnancy complications in humans remains under investigation.

**RITUXIMAB**

Rituximab (RTX) is an anti-CD20 monoclonal antibody that induces rapid and almost complete depletion of CD20+ B cell populations. Originally, approved for the treatment of B-cell lymphomas, RTX has successfully been used in the treatment of systemic autoimmune diseases, including SLE, antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis and rheumatoid arthritis. In contrast to rheumatoid arthritis and ANCA vasculitis, however, two large randomized controlled trials of RTX failed to meet their primary end-points in SLE. Despite these results, RTX continues to be widely used ‘off-label’ for treatment-resistant SLE. RTX use has also been reported in APS, mainly in severe cases with thrombocytopenia or autoimmune haemolytic anemia [42] and in APS patients resistant to standard therapy [43,44]. Erre et al. [43] described 12 patients with primary and SLE-associated APS who were treated with RTX. RTX has also been shown to be an effective therapeutic option for life-threatening Catastrophic APS in a small number of patients [45]. B cells are likely to play a central role in the generation of the aPL-induced clinical manifestations of the disease, so could constitute a therapeutic target in APS. Anecdotally, its use has also been associated with a downregulation of aPL titers [46] and with a reduced rate of recurrent thrombosis in APS patients followed for 10–36 months post-RTX [44].

Very recently, a pilot open-label phase II trial of RTX for noncriteria manifestations of APS (such as thrombocytopenia, skin ulcers, nephropathy and cognitive dysfunction) concluded that RTX may represent a well tolerated option in the therapeutic arsenal for APS. However, it has been reported to be effective in controlling some but not all noncriteria manifestations of APS [47**].

**TISSUE FACTOR INHIBITION**

The upregulation of TF in both immune cells, especially monocytes and the vascular endothelium seems to play a key role in the pathogenic mechanism for aPL-induced thrombosis [48]. Inhibition of TF and associated pathways by which aPL induce TF expression may represent potential therapeutic targets in APS. Currently, some agents have been suggested to induce in-vitro TF inhibition. These include ACE inhibitors, pentoxifylline, an adenosine uptake inhibitor (dilazep), and ss-deoxyribonucleic acid derivatives (e.g. defibrotide) [49]. Novel pharmacologic strategies aiming to inhibit TF upregulation have also been developed, mainly in cardiovascular disease. Several potential mechanisms have been targeted, including TF synthesis inhibition, TF blockade using anti-TF antibodies or recombinant TF pathway inhibitors [50]. In an open-label trial in patients with stable coronary artery disease, Sunol-cH36, a chimeric monoclonal anti-TF antibody exhibited dose-dependent anticoagulant effects inhibiting thrombin formation [51]. The clinical applications of such strategies in APS need to be tested in well designed clinical trials.

**NUCLEAR FACTOR-κB AND P38 MITOGEN-ACTIVATED KINASE INHIBITORS**

The nuclear factor-κB (NF-κB) and the p38 mitogen-activated protein kinase (MAPK) pathways are the major intracellular mechanisms involved in the aPL-induced activation of platelets, endothelial cells and monocytes. In vitro, NF-κB inhibition was associated with a reduction of proinflammatory/prothrombotic biomarkers, including chemokines (CX3CL1 and CCL5) and cytokines (IL-1 and TNFα) [52]. In-vitro reduction of expression of TF was also observed when blocking NF-κB [52]. SB 203580 is an inhibitor of p38α and p38β that suppresses downstream activation of MAPKAP kinase-2 and heat shock protein 27. It has been shown to act at low concentration as a specific p38 MAPK inhibitor, able to significantly reduce the thrombus size and the TF activity in carotid arteries in a mouse model [53]. In a phase I study [54], 24 healthy individuals were exposed to an intravenous dose of LPS preceded 3 h earlier by administration of BIRB 796 BS (another specific p38 MAPK inhibitor), providing preliminary observations of inhibition of coagulation, fibrinolysis and endothelial cell activation. Two randomized, double-blind, placebo-controlled clinical trials investigated the safety, tolerability and efficacy of studies of VX-702, another p38 MAPK inhibitor, in active, moderate-to-severe RA patients [55]. Inhibition of NF-κB or p38 MAPK pathway might
represent a new targeted treatment approach in autoimmune diseases. However, its role in APS treatment needs further investigation.

**COMPLEMENT INHIBITION**

The potential role of complement in the pathogenesis of APS is currently receiving a great deal of attention. There is increasing evidence linking the coagulation and complement cascades, including the findings that human C5 incubated with thrombin generated C5a, and that C5a can trigger the expression of TF [56,57]. Girardi *et al.* found that C5a–C5aR interaction and neutrophils are important mediators of fetal damage in APS. Treatment with heparin, the standard therapy for pregnant patients with aPL, was observed to prevent complement activation and protected mice from pregnancy complications induced by aPL (reviewed in [58]). Mice deficient in complement components C3 and C5 were resistant to enhanced thrombosis and endothelial cell activation induced by aPL. In addition, inhibition of C5 activation by anti-C5 monoclonal antibodies has been proposed to reduce aPL-mediated prothrombotic status [59]. In a mouse model of APS, a C5a receptor antagonist was observed to induce a reduction on aPL-mediated cellular effects, including TF expression [60]. The effective use of eculizumab, a humanized monoclonal IgG2/4 antibody that binds the complement protein C5, preventing cleavage into C5a and C5 has been reported in severe cases of APS, such as the catastrophic variant of the syndrome [61–63]. These findings emphasize the importance of developing and testing complement inhibition therapies in patients with APS. The Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Antibody Syndrome and Systemic Lupus Erythematosus (PROMISSE) [64] is currently evaluating biomarkers that predict pregnancy morbidity and will potentially stimulate interventional trials of complement inhibition in patients at risk of aPL antibody-associated clinical manifestations [65].

**BLOCKING OF ANTIPHOSPHOLIPID ANTIBODY/β2GPI RECEPTORS ON TARGET CELLS**

The interaction of aPL with several cell surface receptors, including annexin A2 and Toll-like receptor 4 (endothelial and monocytes), lipoprotein receptor-related protein family (platelets) and C5a receptor (neutrophils) has been investigated as potential targets to prevent aPL-induced thrombosis. Evidence indicates that annexin A2, a receptor for tissue plasminogen activator and plasminogen, binds β2GPI on target cells. Interestingly, an anti-A2 monoclonal antibody significantly decreased aPL-induced expression of thrombophilic molecules on cultured endothelial cells, indicating that blocking A2 might reduce the pathogenic effects of aPL in inducing thrombosis [66,67]. Pierangeli and co-workers [68] investigated the role of TIFI, a 20 amino acid synthetic peptide that shares similarity with the domain V of β2GPI in APS. They observed the ability of TIFI to reduce aPL-mediated thrombosis in mice by competing with β2GPI and preventing its binding to target cells. Inhibition of aPL binding to the receptor proteins on target cells might be important in designing new modalities for the treatment of thrombosis in APS focusing on immunomodulatory option.

Several independent groups have demonstrated that polyclonal IgGs derived from the patients with APS bind domain I of β2GPI [69–71]. Therefore, antidomain I antibodies are likely to represent an important subpopulation of pathogenic anti-β2GPI antibodies. Theoretically, molecules that inhibit binding of these aPLs to native domain I may be useful as therapeutic agents in patients with APS. This hypothesis has been proposed and preliminary results showed that a recombinant D1 molecule, and a recombinant mutant D1 with enhanced aPL binding properties, may be used as an inhibitor of aPL binding, and thus inhibit aPL-induced pathogenicity [72].

**INTRAVENOUS IMMUNOGLOBULINS**

The use of intravenous immunoglobulins (IVIG) in obstetric APS refractory to standard treatment has currently failed to be implemented widely because of its lack of evidence of efficacy, expense and supply shortages [73]. Studies [74,75] have failed to observe statistically significant improvements in obstetric and neonatal outcomes with the use of IVIG in combination with aspirin and low molecular weight heparin (LMWH), when compared with aspirin and LMWH only regimens. Consequently, IVIG is often reserved for patients that are refractory to LMWH or when additional indications such as autoimmune thrombocytopenia are present.

More recently, two studies [76,77] showed the efficacy of IVIG in addition to conventional therapy, in primary and secondary APS patients, in preventing the occurrence of further thromboembolic events. However, further clinical studies on a larger group of patients are necessary to fully understand the mechanisms of action and the optimal doses of IVIG in thrombotic APS.
AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

Autologous hematopoietic stem cell transplantation (HSCT) is currently being evaluated as a treatment for autoimmune diseases [78]. In 2005, Statkute et al. [79] suggested that autologous HSCT might be performed safely in patients with APS and appeared to be effective therapy for eliminating aPL and preventing thrombotic complications in patients with SLE. However, few cases of HSCT in APS have been reported [80,81] and long-term follow-up data are required before this option could be considered a potential therapeutic approach for the syndrome.

CONCLUSION

In the long-term management of APS patients, controlled studies with vitamin K antagonist alternatives, such as the new anticoagulant agents (oral direct and indirect thrombin inhibitors) are essential. Newer therapeutic agents targeting pathways involved in the development of aPL-mediated clinical manifestations are under investigation. However, the multifactorial mechanisms underlying thrombosis and pregnancy morbidity in APS are still not fully understood and this might limit the development of new targeted therapies for APS. Potentially, the current ‘antithrombotic’ approach to APS patients will be replaced in the future by an ‘immunomodulatory’ approach as our understanding of the mechanisms of aPL-mediated clinical manifestations improves.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

■ of outstanding interest
