12

Antiphospholipid Syndrome – An Evolving Story of a Multisystemic Disease

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1. Introduction

Antiphospholipid syndrome (APS) is an autoimmune multisystemic disorder characterized clinically by recurrent thrombosis and pregnancy morbidity and serologically by the presence of antiphospholipid antibodies (aPL) including anticardiolipin (aCL) and anti-β2 glycoprotein I (anti-β2GPI) antibodies and lupus anticoagulant (LA) [1].

Historically, aPL antibodies were classified based on the clinical laboratory test in which they were detected, i.e. LA and aCL antibodies. It is now widely accepted that aPL antibodies are a heterogenous group of antibodies that react with a myriad of phospholipids (PLs), PL-protein complexes and PL binding proteins. The main antigenic target of these antibodies is recognized to be $\beta_2$glycoprotein I (β_2GPI), which along with prothrombin accounts for more than 90% of the antibody binding activity in APS patients. Other potentially significant antigenic targets include tissue plasminogen activator (tPA), phosphatidylserine (PS), plasmin, annexin 2, activated protein C (APC), thrombin, antithrombin (AT) and annexin A5 [2,3].

In the general population, APS is the most common cause of acquired thrombophilia and is a recognized risk factor for the development of deep vein thrombosis (DVT) with or without pulmonary embolism, new strokes in individuals below the age of 50 and recurrent fetal loss [4]. The prevalence of DVT occurrence in the general population is estimated at 2-5%, 15 - 20% associated with APS, suggesting that the prevalence of venous thrombosis associated with APS may be as high as 0.3-1% of the general population [4]. APL antibodies are present in 30-40% of systemic lupus erythematosus (SLE) patients and up to a third of these patients (10-15% of SLE patients) have clinical manifestations of APS, especially venous or arterial thromboses [5,6].

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The APS related thrombotic events range in severity from the relatively benign superficial thromophlebitis to myocardial infarction, stroke and catastrophic APS (CAPS) [7]. APS also accounts for a significant proportion of recurrent pregnancy loss in SLE patients, indeed, aPL are now regarded as the most frequent acquired risk for a treatable cause of recurrent pregnancy loss and for pregnancy complications (early and severe pre-eclampsia) [5,8].

The first description of aPL antibodies dates back to 1952, when Moore *et al* described patients suffering from SLE with a persistently false positive VDRL flocculation test for syphilis, a test based on the detection of antibodies against cardiolipin (CL) extracted from beef heart [7]. In the same year, Conley et al [8,9] described two SLE patients with a peculiar circulating inhibitor of coagulation [10]. These "anticoagulants" could inhibit *in vitro* coagulation assays, but did not influence the activity of coagulation factors and were not associated with a bleeding diathesis. Feinstein and Rapaport introduced the term LA to describe this phenomenon in 1972 [10]. Although the relation between thrombosis and the presence of these anticoagulants in SLE patients was already noticed in 1963 [11], it took until 1980 before the association between LA and thrombosis was widely recognized [12]. As LA was found to be associated with a persistently false positive syphilis test, this led to the development of an aCL immunoassay and the establishment of the association between thrombosis and aCL anticardiolipin [13]. From this time on, patients presenting with thrombosis and/or pregnancy loss in combination with persistently positive aCL antibodies and/or circulating LA were considered to have the APS [14,15]. Subsequently, patients with systemic lupus erythematosus (SLE) and related connective tissue diseases (CTD) that had abnormal LA tests, were labeled as 'secondary' APS (SAPS) in the presence of these conditions and 'primary' (PAPS) in their absence [16]. A study of patients with SLE showed that aCL positivity preceded the onset of a more severe form of SLE, as well as SLE complicated with thrombosis, pregnancy loss and thrombocytopenia [5]. However, studies have found no difference between PAPS and SAPS with respect to the clinical complications, the timing of those complications, the prognosis or frequency of positive aCL, LA or other autoantibody tests. In addition, management of PAPS and SAPS is the same and prognosis does not appear to differ [17].

2. Traditional and non-traditional manifestations of APS

APS is classically characterized by vascular thromboses or obstetric morbidity in association with the presence of aPL antibodies [1]. Vascular thromboses include venous thromboses resulting clinically in deep venous thrombosis and/or pulmonary emboli while arterial thromboses may present with ischemia affecting limbs, cerebral vascular accidents or transient ischemic attacks and small-vessel thrombosis may result in cutaneous ulceration [1,18]. Presence of thrombosis should be confirmed with a diagnostic angiogram, Doppler ultrasound, pulmonary scintigraphy, histopathology or computed tomography (CT) or magnetic resonance imaging (MRI) of the brain depending on the clinical context [1].

In a longitudinal cohort of patients with APS, transient ischemic attacks (TIA)s and cerebrovascular accidents (CVA)s were the most common thrombotic events occurring in 2.4% and 2.3% respectively of patients with established APS followed by pulmonary embolism and deep venous thrombosis over a period of 5 years [18].

Obstetric manifestations of APS include fetal loss with loss after 10 weeks of gestation being more strongly associated with APS, placental insufficiency potentially resulting in

decreased gestational weight or fetal distress and preterm delivery and development of pre-eclampsia and frank eclampsia [1]. Early pregnancy loss occurs in 17.1% and late pregnancy loss occurs in 6.7% of pregnancies in women with established APS while 35% of successful pregnancies were premature and 13.7% had intrauterine growth restriction [18].

Catastrophic APS (CAPS) is the rare but life-threatening development of wide-spread intravascular thrombosis seen in less than 1% of patients with APS [18-20]. Patients present acutely with multi-organ system failure, evidence of small vessel thrombosis and presence of positive aPL antibodies [19,20]. Death occurs in approximately 45% of patients during the acute event with primary causes being cerebral involvement, cardiac involvement, infections, multiorgan failure, pulmonary involvement and abdominal involvement [20]. Infection is the most common trigger identified in CAPS being present in approximately 20% of patients [20].

Patients with APS may also develop manifestations not included in the classification criteria. Neurologic symptoms other than strokes or TIAs including chorea, dementia, transverse myelitis, multiple sclerosis and epilepsy have been attributed to APS although studies are contradictory [1,18,21]. Livedo reticularis occurs more commonly in APS and may progress to livedo vasculitis with purpuric lesions, nodules and painful ulcerations [1,18,22]. Presence of livedo reticularis appears to carry an increased risk of arterial thrombosis, CVA and pregnancy loss [22].

Thrombocytopenia is the most common hematologic manifestation, occurring in over 30% of patients with APS [22]. Cardiac involvement frequently manifests as valvular disease with presence of mitral or tricuspid valve thickening or regurgitation and presence of valvular vegetations [19]. APS is also associated with a thrombotic microangiopathy of the renal arterioles and glomeruli known as APS nephropathy, which leads to hypertension, nephrotic range proteinuria, hematuria and progressive renal insufficiency [1].

3. Current diagnostic algorithms

a. "Criteria" aPL tests.

Current classification criteria for definite APS require the use of three "standardized" laboratory assays to detect aPL antibodies. These assays include aCL, both IgG and/or IgM by enzyme-linked immunosorbent assay (ELISA), the anti-β_2GPI IgG and/or IgM by ELISA and the LA [1]. These tests, when positive, represent criteria for diagnosis when at least one of the two major clinical manifestations (thrombosis or pregnancy losses) is present according to the revised Sapporo criteria (Table 1).

Laboratory testing for aPL antibodies is one of the most problematic areas in the field of APS. The confirmation of diagnosis of the APS relies on laboratory tests, since clinical manifestations such as thrombosis and pregnancy losses may occur for many reasons not related to the presence of aPL antibodies. Most importantly, patients with APS who have experienced thrombosis and/or pregnancy losses need a specific therapy that is often life-long and must be personalized, requiring careful monitoring of additional risk factors to prevent recurrences of APS manifestations. Given the potential serious side effects of anticoagulant therapy, a solid diagnosis is essential in planning management.

a)Lupus anticoagulant (LAC)	Positive on two or more occasion at least 12 weeks apart, detected according to the guidelines of ISTH
b)Anticardiolipin (aCL) antibody	Positive for IgG or IgM isotype in serum or plasma, present in medium and high titer on two or more occasions, at least 12 weeks apart, measured by standardized ELISA.
c)Anti-β_2GPI antibody	Positive for IgG or IgM isotype (in titer > the 99th percentile) on two or more occasions, at least 12 weeks apart measured by standardized ELISA

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Table 1. Laboratory Criteria for APS (Revised Sapporo Criteria).

Although international consensus guidelines for the determination of LA have been published and revised, the existence of "standardized" tests for detection of aCL and anti-β_2GPI has remained elusive. Furthermore, despite over 7000 publications related to the clinical use of aPL antibody tests, a consensus on clinical recommendations has been difficult to achieve. This difficulty appears related to sub-optimal design in clinical studies and a lack of laboratory standardization in areas such as the following: 1) units of measurement, 2) calibration curves, 3) determination of cut-off values, and 4) laboratories not performing the tests according to established guidelines. Significant inter-assay and inter-laboratory variation in the results of both aCL and anti-β_2GPI testing still exists, affecting the consistency of the diagnosis of APS [23].

Over the years, international workshops have worked hard to standardize the laboratory test in this area. These workshops include the APL European forum, the Australasian Anticardiolipin Working Party (AAWP), the College of American Pathologists (CAP), the National External Quality Assessment Scheme (NEQAS), and the Standardization Subcommittee (SSC) on lupus anticoagulant and phospholipid-dependent antibodies of the International Society of Thrombosis and Hemostasis (ISTH). While some laboratories can obtain reliable testing results, there is still wide inter-laboratory variation despite all the efforts at standardization. This situation may result from laboratories performing aPL assays with their own protocols or using commercial kits that do not conform to the proposed guidelines for these tests. Furthermore, standardization of tests or re-evaluation of standardization is important since APS is related to serious complications like thrombosis and pregnancy loss; missing a diagnosis because of laboratory variability could have serious medical consequences. The use of semi- or fully-automated analyzers and commercial kits instead of in-house assays poses additional challenges to the process of standardization [23].

To address the challenges on aPL testing described above, an international "*Criteria aPL Task Force*" (Task Force) of researchers and scientific leaders in the field was formed prior to the 13th International Congress on Antiphospholipid Antibodies in Galveston, TX, April 2010 (APLA 2010). The "Criteria" aPL Task Force was further divided into three subgroups, which were charged by the APLA 2010 Congress Chair to address, in an evidence-based manner, various topics related to the testing of aCL, anti-β_2GPI and LA. To accomplish its mission, the Criteria aPL Task Force considered published information, the results of a survey distributed among APLA 2010 congress attendees and the discussions that occurred during a special preconference workshop at APLA 2010. On the basis of this approach, the

Task Force reached several conclusions and proposed recommendations discussed below and summarized in Table 2; this information has recently been published [24-26].

Subgroup1 International Consensus Guidelines on assay performance of aCL and anti-β_2GPI assay.	**Conclusions** Development of International Consensus Guidelines for aCL and anti-β_2GPI assays including pre-analytical, analytical and post-analytical considerations. *(Lakos G et al. Arthritis Rheum 2011 Sep 27. doi: 10.1002/art.33349)*
Subgroup 1 Guidelines on use of calibrator for aCL/ anti-β_2GPI assays and selection and preparation of reference material	**Conclusions:** a) Tests to be reported in GPL/MPL units if monoclonal antibodies are used as a calibrator. b) Levels of secondary calibrators should be meticulously defined prior to use. c) Evaluation of the performance of various monoclonal and polyclonal antibodies in order to identify optimal material for standardization. d) Establishment of international units for measurement for anti-β_2GPI antibodies (work in progress) *(Pierangeli S et al. Clin Chim Acta. 2011 Oct 15. [Epub ahead of print])*
Subgroup 2 Review of the Updated ISTH SCC guidelines on the use of Lupus anti-coagulant (LAC) for diagnosis.	**Conclusions:** a) Weak LAC does not predict behavior in vivo. b) Consideration of false-positive results with the use of phospholipid –diluting agent. c) Additional lab testing to differentiate LAC from factor inhibitors, if clinically indicated. d) An inter-laboratory study to validate the statement about integrated test and not requiring performance of "mixing" tests e) LAC to be tested 2-3 weeks after warfarin discontinuation f) Clinicians to contact reference laboratories to discuss specific issues related to LAC and results interpretation.
Subgroup 3 Role of aPL as "risk factors".	**Conclusions:** a) Develop collaborations with existing large, population-based, prospective cohorts with available data on thrombosis and/or pregnancy outcomes to examine the value of aPL-SCORE. b) Full panel of currently available aPL test should be performed and if possible new tests like anti- PS/PT, anti-β_2GPI domain I, annexin A5 should also be evaluated

Table 2. "CRITERIA" aPL Task Force Recommendations.

b. "Non-Criteria" aPL Tests.

As indicated above, the revised classification criteria for the diagnosis of APS include the positivity of at least one of the three 'Criteria' aPL tests [1]. However, the use of these tests

may not guarantee full sensitivity and specificity to confirm a diagnosis of APS. In clinical practice, there are indeed many 'false positives' with aPL tests, especially the aCL ELISA, which can give positive results in clinical conditions besides APS; these conditions include infectious disease (i.e., syphilis), malignancy and other autoimmune diseases. On the other hand, there are patients with a clinical pattern strongly suggestive of APS, but persistently negative for 'Criteria' tests. In addition the "criteria" aPL tests may not identify the "pathogenic" subpopulations of aPL.

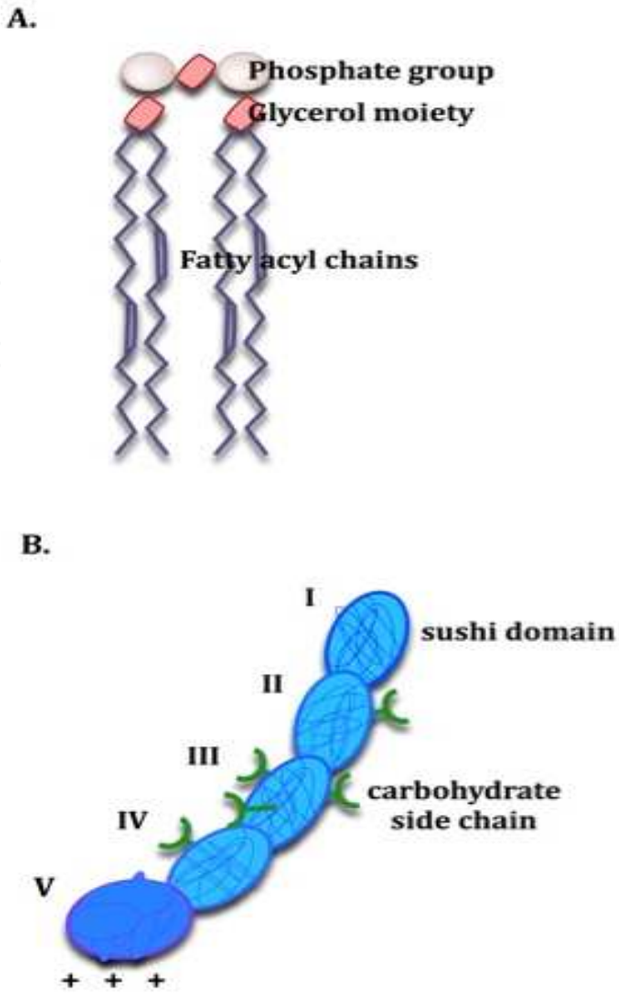
Several autoantibodies have been demonstrated to bind directly to negatively charged phospholipids other than CL (individually or as a phospholipid mixture) or to other proteins in the coagulation cascade (i.e., prothrombin and/or phosphatidylserine-prothrombin complexes); antibodies can also interfere with anticoagulant activity of the annexin A5. However, the clinical and diagnostic utility of these newly developed assays as well as their standardization requires much further study. In some cases, these new assays lack standardization and there are not international units of measurements.

A "Non-Criteria" aPL Task Force assembled prior to APLA 2010 was charged by the Congress Chair to address, in an evidence-based manner, the status of various new tests being developed for confirmation of diagnosis of APS. The results and recommendations of that task force have been recently published elsewhere [27].

4. Antigenic targets of antiphospholipid antibodies: Phospholipids and phospholipid binding proteins

As stated previously, aPL represent a heterogenous group of antibodies with reactivity to not only PLs but also proteins, in particular those able to bind and form complexes with PLs [2]. Historically, serological activity against cardiolipin (CL) (Figure 1a), an anionic PL found in mitochondrial membranes, was one of the earliest key descriptive features of APS and although still important is overshadowed by β_2GPI, which is now recognized as the main antigenic target of pathogenic aPL [2]. Indeed, β_2GPI along with prothrombin (PT) account for more than 90% of the antibody binding activity in APS patients and it is unsurprising that antibodies against these 2 abundant proteins involved in hemostasis are most consistently associated with LA activity [28]. β_2GPI consists of five contiguous domains (Figure 1b), the first proposed to be the binding site for pathogenic anti-β_2GPI antibodies and the fifth the binding site for anionic and hydrophobic phospholipids such as phosphatidylserine (PS), lyso (bis) phosphatidic acid (LBPA), and CL exposed on cell surfaces and protein receptors [2,29]. The role that apoptosis plays in the exposure of these phospholipids on the cell surface and the subsequent interaction with β_2GPI has been proposed as a possible mechanism for the production of pathogenic aPL in APS patients [30]. An interesting pathogenic role for oxidized low-density lipoprotein (ox-LDL)/β_2GPI complexes bound by aPL in the initiation and progression of atherogenesis has been described [31,32].

Several models have been put forward for pathogenic anti-β_2GPI Abs complexed with β_2GPI activating monocytes, ECs, trophoblasts and platelets via simultaneous binding to PLs and candidate protein receptors to induce production of tissue factor and proinflammatory cytokines [33-36]. *In vivo* and in *in vitro* studies have demonstrated the role of annexin A2 (AnnA2), in association with Toll-like receptor 4 (TLR4) and/or apolipoprotein ER2'



Pierangeli et al.

Fig. 1. Schematic representation of Cardiolipin and of β_2Glycoprotein I.
A. Cardiolipin structure contains 2 negatively charged phosphate head groups, 3 glycerol moieties and 4 fatty acyl chains
B. β2-glycoprotein I structure consists of 5 contiguous sushi domains. The first 4 consisting of 60 amino acids and the fifth consisting of 80 amino acids and more positive charged amino acids

(ApoER2′) that act as co-receptors containing intracellular signaling domains, in the activation of ECs, monocytes and cells of the decidua and trophoblast [37-39]. Candidate receptors on platelets include ApoER2′ and the glycoprotein Ibα (GPIbα) subunit of the GPIb-V-IX receptor and Sikara et al have demonstrated a putative role for platelet factor 4 (PF4) in the stabilization and binding of dimeric β_2GPI /anti-β_2GPI complexes to platelet membranes [40,41].

Many serine proteases that function in maintaining hemostasis are targets of autoantigens in APS patients. These include activated protein C (APC), prothrombin, antithrombin (AT) and many coagulation factors including factors IXa, IIa and II [42]. There is evidence to suggest

that antibodies directed against AnnA5, an abundant cationic protein that functions as a natural anticoagulant especially in placental tissue, can cause placental thrombosis and fetal resorption in mice, although there is conflicting evidence of the significance of these antibodies in APS patients [43,44]. A recently described protein antigenic target, vimentin, has been suggested to play a putative role in platelet and leukocyte activation in APS patients but further characterization of the role of this cytoskeletal protein is necessary [45].

5. Origins of APS: Infection-associated APS and molecular mimicry

The failure of normal T cell tolerance mechanisms seems to be an important component for the development of autoimmunity in several diseases. In APS, there is evidence to suggest that *molecular mimicry* can induce production of pathogenic APL antibodies, presumably because of a breakdown in normal peripheral tolerance mechanisms [46]. Although aPL were first characterized by their ability to bind CL, it is now well accepted that these antibodies recognize various PL and protein antigenic complexes [1,2].

Indeed, efforts to induce high titer production of pathogenic aPL in animal models succeeded only after immunization with heterologous β_2GPI rather than pure phospholipids [47]. This led researchers to believe that perhaps *in vivo* binding of foreign PL-binding proteins resembling β_2GPI to self phospholipids in APS patients may lead to the formation of immunogenic complexes, against which aPL antibodies are produced. Gharavi *et al* in 1999 synthesized a 15 amino acid peptide, GDKV, which spanned an area of the fifth domain of β_2GPI known to be a major PL-binding site of the molecule, and demonstrated the peptide's ability to induce pathogenic aPL and anti-β_2GPI antibody production in mice [48]. A monoclonal antibody with aPL and anti-β_2GPI activity generated from these GDKV-immunized mice was shown to be pathogenic using *in vivo* models for thrombus enhancement and microcirculation [49]. The resulting search for candidate peptides in microorganisms that exhibited functional and sequence similarity to the PL-binding domain of β_2GPI produced the peptides TIFI and VITT from cytomegalovirus (CMV), TADL from adenovirus (AdV) and SGDF from *Bacillus subtilis*. All these peptides had strong similarities with GDKV and induced high titers aPL and anti-β_2GPI production in mice. Subsequent *in vivo* and *in vitro* experiments confirmed the pathogenicity of antibodies induced in TIFI-immunized mice [50-52].

Further supporting evidence for molecular mimicry as a possible mechanism for APS development was provided by a study evaluating the APS-related pathogenic potential of microorganisms carrying sequences related to a hexapeptide, TLRVYK, known to be specifically recognized by a pathogenic monoclonal anti-β_2GPI Ab [53]. Following immunization with *Haemophilus influenzae*, *Neisseria gonorrhoeae* or tetanus toxoid; high titers of antibodies of anti-peptide (TLRVYK) and anti-β_2GPI activity were observed in BALB/c mice. These affinity-purified antibodies were then infused into naive mice at day 0 of pregnancy. At day 15, these mice had significant thrombocytopenia, prolonged activated partial thromboplastin times (aPTT) and increased frequency of fetal loss compared to controls [53].

Infections are thought perhaps to be the most prominent environmental trigger for aPL production and APS development. Syphilis was the first infectious disease recognized to be linked to aPL production and this infectious type aPL is for the most part non-pathogenic

[54]. However, several subsequent reports have shown that many other infections not only trigger aPL production but are associated with the development of APS manifestations as well [55,56]. CMV, parvovirus B19, Human immunodeficiency virus (HIV), Hepatitis B and C viruses, Human T-cell lymphoma/leukemia virus (HTLV) and Varicella Zoster Virus (VZV) are just a few of the infectious agents that have reported associations with aPL production and APS manifestations [56]. A recent study has demonstrated that protein H of *Streptococcus pyogenes* can bind β_2GPI, inducing conformational changes, exposing hidden epitopes and in so doing then enable production of anti-β_2GPI antibodies [57].

Rauch *et al* have recently put forward a hypothesis regarding the dual role of the innate immune system in the initiation and progression of APS based on their work. This hypothesis highlights the central part played by toll-like receptors (TLRs), especially TLR4, in inducing a break in tolerance, aPL production and epitope spread to several autoantigens [58]. Utilizing lupus prone mice treated with CMV derived peptides in the presence of TLR7 or TLR9 agonists and other lupus prone mice deficient in TLR7 or both TLR7 and TLR9, our group has demonstrated for the first time that both these TLRs are involved in aPL production in β_2GPI immunized mice [59].

6. Genetics of APS

Animal models and family and population studies have been used to highlight genetic associations with APS disease characteristics and the occurrence of aPL antibodies in patients. In 1992 Hashimoto *et al* described an animal model of lupus associated APS in NZW x BXSB F1 (W/B F1) male mice that displayed spontaneous production of IgG aCL antibodies which exhibit co-factor (β_2GPI) dependent binding to cardiolipin [60]. Interestingly, analysis of the genes utilized in the production of pathogenic aCL in these mice showed preferential usage of certain V_H (variable region of heavy immunoglobulin chain) and V_K (variable region of kappa light immunoglobulin chain) genes, whereas other non-pathogenic aCL utilize random V gene combinations possibly indicating that pathogenic aCL production in these mice is antigen driven rather than germiline encoded [61]. Genome-wide analysis using microsatellite markers in these mice and their progeny revealed that the generation of each disease character was controlled by two independently segregating major dominant alleles producing full expression as a complementary gene action. Although there was complete genetic concordance between the occurrence of antiplatelet Abs and thrombocytopenia, other disease characteristics were independently controlled by different combinations of two dominant alleles suggesting that no single genetic factor can explain the pathogenesis of APS [62]. Papalardo et al have recently shown, using MHCII deficient mice and MHCII deficient mice transgenic for human MHCII haplotypes, that MHCII is necessary for producing aPL after immunization with β_2GPI and certain haplotypes are more effective than others [63].

Since 1980, several studies have described families with high incidences of primary APS associated with LA, aCL and other autoantibodies [64-66]. The most consistent HLA associations in families with APS are HLA-DR4 and DRw53; other less consistent associations include DR7, DQw3, DQw7, A30, Cw3 and B60 [67-70]. In non-familial population studies HLA-DR4 and DRw53 were also consistently associated with APS disease characteristics in addition to DR7 and DQB1*0302 [71-73].

The occurence of aCL antibodies has been reported in association with DRB1*09 in Japanese patients with APS secondary to SLE and with C4A or C4B null alleles in black American populations. However, patients in the Hopkins Lupus Cohort who were homozygous for C4A deficiency had a lower frequency of aCL and LA than patients without this deficiency [74-76]. Other less consistent non-familial HLA associations with APS include DRB1*04, DQB1*0301/4, DQB1*0604/5/6/7/8/9, DQA1*0102 and DQA1*0301/2 [73,77-79]. Several non-HLA genes associations with increased autoantibody production and risk of thrombosis have been described in APS patients. Perhaps the most profound is a polymorphism in domain 5 of β_2GPI, valine instead of leucine at position 247, which is found more frequently in patients with APS than matched controls and is associated with anti-β_2GPI production and increased risk for arterial thrombosis in these patients [80]. Other less established genetic associations with increased thrombosis in APS include the factor V Leiden mutation, the G20210A prothrombin mutation (F2 G20210A) and protein C and S deficiencies [81].

7. Pathogenic effects of antiphospholipid antibodies: What we have learned from *In Vivo* animal models?

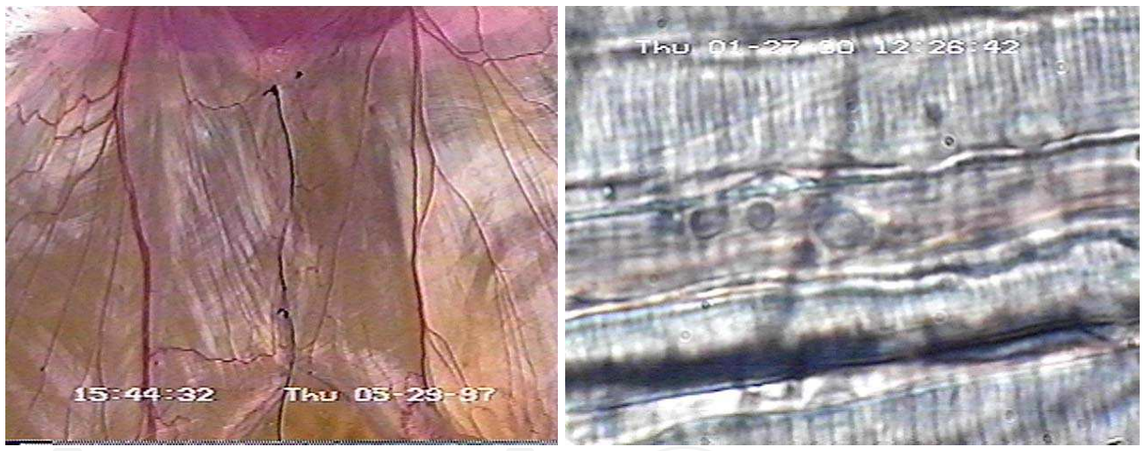
a. Animal Models of Thrombosis and Endothelial Cell Activation

Based on the observation that patients with aCL antibodies appear to get thrombi at intermittent intervals, our group hypothesized that these antibodies might only enhance the thrombotic process after another inciting agent initiated it. With this in mind, Pierangeli *et al* [82] turned to a mouse model of thrombosis devised by Stockmans *et al* [83] and modified by Barker *et al* [84] that enables measurement of the dynamics of thrombus formation after this is induced by a standardized injury. In a series of experiments, this group of investigators found that CD1 male mice, injected with purified immunoglobulins (4 IgG, 3 IgM, 2 IgA preparations) or with affinity purified aCL antibodies (2 IgG and 2 IgM) had significantly larger and more persistent thrombi compared to mice immunized with immunoglobulins from healthy humans. The effect of these Ig preparations was also dose-dependent [85]. In collaboration with Dr Pojen Chen (UCLA, Los Angeles, Ca), the group showed also that human monoclonal aCL antibodies derived from a patient with the APS had thrombogenic properties *in vivo* [86]. Similarly, mice producing aCL antibodies after immunization with β_2GPI or human aPL antibodies also had thrombogenic properties *in vivo* [87]. Furthermore, murine monoclonal aPL and a monoclonal antibody obtained by immmunization with the phospholipid-binding domain of β_2GPI, also showed thrombogenic properties in their model [48]. The results of studies utilizing this model showed for the first time that aPL antibodies significantly enhance thrombus formation in mice.

Subsequently, Jankowski *et al* and Fischetti *et al* demonstrated the thrombogenic effects of monoclonal and polyclonal anti-β_2GPI in hamsters and rats respectively [88,89]. More recently, Arad and colleagues showed in an animal model that affinity purified anti-β_2GPI antibodies induce thrombosis in mice in a dose-dependent manner [90]. Hence, several investigators have underscored and confirmed the causal relationship between the presence of these autoantibodies and thrombo-embolic complications. In all these models, a priming effect (injury, endotoxin, etc.) was needed to induce thrombus formation in addition to aPL antibodies injected passively into the animals or induced by active immunization. Not only did these models demonstrate "enhanced" thrombus formation compared to controls but

also they mimicked what happens in actual APS patients, in whom thrombus formation follows a triggering event (trauma, immobilization, infection, etc).

Antibody mediated endothelial cell activation and injury have been identified as potential factors that may be involved in the pathogenesis of thrombosis by aPL. The relationship between endothelial cell activation and the thrombotic diathesis in APS could be explained by a procoagulant state of the activated endothelium or by the adherence of mononuclear cells accompanied by the increased expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin (E-sel). Pierangeli *et al* have utilized a unique animal model of microcirculation that allows one to examine and measure changes in adhesiveness of leukocytes in the microcirculation of an isolated cremaster muscle in mice [34], as an indication of EC activation *in vivo* (Figure 2). These parameters include rolling and sticking of leukocytes and diapedesis of white blood cells into the tissue from the blood vessel, etc. Utilizing this model, those investigators first showed that polyclonal aPL antibodies significantly enhance adhesion of leukocytes to endothelium *in vivo* and that this correlated with enhanced thrombosis [34,91]. These effects were observed utilizing some human and some murine monoclonal aPL antibodies [92].



A. B.

Pierangeli et al.

Fig. 2. In Vivo Model of Endothelium Activation. Mice are injected with aPL antibodies or control immunoglobulin twice at 0 and 48 hours. At 72 hours the cremaster muscle of anesthetized mice (A) is isolated and the adhesion of leukocytes in 5 postcapillary venules is assessed (B). Adhesion is defined as leukocytes that remained stationary for at least 30 seconds.

In summary, these animal models of thrombosis and endothelial cell activation have not only been useful in demonstrating the pathogenic effects of aPL antibodies and their causative role in inducing APS morbidity, but have also been instrumental in dissecting the intracellular mechanisms involved, in identifying cellular receptors activated by aPL antibodies *in vivo* and in testing potential new treatments for APS (discussed in detail in other sections of this chapter) [37,39,51,93-105].

b. Animal Models of Pregnancy Loss in APS.

Considerable progress has also been made in developing an *in vivo* model of pregnancy loss related to aPL antibodies in the last 20 years. Gharavi *et al* first reported that MRL/lpr mice with IgG aCL antibodies had smaller litter sizes than controls [106]. However, these lupus prone mice produce autoantibodies with multiple specificities and have other clinical abnormalities (such as kidney disease), which may account for pregnancy loss.

In 1990, Branch *et al* reported that Balb/c mice passively immunized with immunoglobulins from patients with the APS had nearly 100% fetal wastage compared to mice passively immunized with immunoglobulins from patients with normal human immunoglobulins [107]. Subsequently, experiments demonstrated that passive immunization of mice with polyclonal or monoclonal IgG aCL antibodies resulted in significant fetal resorption [108]. Furthermore, Gharavi and colleagues showed that if aCL antibodies are induced in PL/J mice (autoimmune prone mice) the animals showed an increase rate of fetal resorption [109].

Fishman and colleagues reported that production of IL-3 and GM-CSF is decreased in splenocytes derived from their mouse models [110] and that intra-peritoneal administration of recombinant IL-3 to pregnant mice resulted in abrogation of fetal loss and thrombocytopenia.

More recently Girardi *et al* utilized that mouse model to demonstrate the involvement of complement activation in aPL-mediated pregnancy morbidity utilizing various mice deficient in complement components [96,111]. Furthermore, that group of investigators showed that heparin prevents pregnancy loss in mice injected with aPL due to the complement inhibitory properties of the drug and not to its anticoagulant effects [112].

8. Direct proinflammatory and prothrombotic effects of antiphospholipid antibodies on platelets, monocytes and endothelial cells

The activation of platelets, endothelial cells and monocytes via direct binding of aPL antibodies plays an important role in the creation of a proinflammatory and prothrombotic phenotype in APS patients. Binding of dimeric β_2GPI/anti-β_2GPI complexes to platelets is dependent on exposure of anionic phospholipids, especially phosphatidylserine (PS), on platelets, which occurs after stimulation by agonists such as thrombin, collagen, and adenosine diphosphate (ADP) [113,114]. Pathogenic aPL enhance the expression of GPIIb/IIIa, a major fibrinogen receptor, on platelets and our group has shown that in GPIIb/IIIa deficient (β_3-null) mice and mice treated with a monoclonal anti-GPIIb/IIIa antibody there is reduced aPL-mediated thrombus formation [115,116]. Our group has also demonstrated that the major intracellular signaling pathway activated by aPL binding to platelets is the p38 mitogen activated protein kinase (MAPK) pathway and that subsequent phosphorylation of cytosolic phospholipase A2 (cPLA2) results in thromboxane B2 (TXB2) production. After initial activation through the p38 MAPK pathway, other MAPK pathways in platelets, such as ERK-1 (p44 MAPK) and ERK-2 (p42 MAPK), have a potential secondary role in signaling [116].

The adhesion molecules VCAM-1, ICAM-1 and E-sel have been shown to be upregulated in ECs activated by aPL [34,117,118]. Utilizing ICAM-1, VCAM-1, E-sel and P-selectin (P-sel) knockout mice, our group demonstrated the importance of ICAM-1, E-sel, P-sel and VCAM-

1 in promoting leukocyte adhesion and thrombus formation mediated by human polyclonal and monoclonal aPL antibodies [91]. Many groups have also demonstrated the upregulation of tissue factor (TF) expression and micro-particle formation with associated increases in interleukin-6 (IL-6) and IL-8 secretion in ECs and monocytes treated with aPL [119-122]. López-Pedrera *et al.* showed that aPL could induce TF in monocytes by activating the phosphorylation of mitogen-activated protein kinase/extracellular regulated kinase (MEK-1/ERK) protein, and the p38 mitogen-activated protein kinase (MAPK)-dependent nuclear translocation and activation of nuclear factor kB (NFkB)/Rel proteins [123]. Increased surface expression of both vascular endothelial growth factor (VEGF) and Flt-1 on monocytes and elevated plasma levels of VEGF in APS patients suggests that TF upregulation in monocytes may occur as a result of stimulation of the Flt-1 tyrosine kinase receptor by VEGF [33]. Many researchers have provided evidence that upregulated TF mRNA and antigen expression and TF pathway activation plays a key role in APS thrombotic manifestations. Indeed, our group found in an ongoing clinical trial, that mean serum levels of soluble TF, tumor necrosis factor-α (TNFα) and VEGF were significantly elevated in APS patients compared to controls and treatment with fluvastatin, a statin with efficacy in treating APS, resulted in significant decreases of these pro-inflammatory markers in most APS patients [124].

Role of complement in aPL-mediated thrombosis.

Complement inhibitors are now being tested in patients with inflammatory, ischemic and autoimmune diseases [125-127]. The C5 component of complement is cleaved to form products with multiple proinflammatory effects and thus represents an attractive target for complement inhibition in immune-mediated inflammatory diseases. Furthermore, C5a is the most potent anaphylotoxin and a powerful chemotaxin for neutrophils and monocytes, with the ability to promote margination, extravasation and activation of these cells. In addition, C5b-9 can also stimulate the release of multiple proinflammatory molecules and may well play an important role in inflammation apart form its lytic function. Thus, blocking C5b-9 as well as C5a generation may be required for optimal inhibition of the inflammatory response.

At the same time, inhibition of the complement cascade at the level of C5 does not impair the generation of C3b through the classical and alternative pathways, preserving C3b-mediated opsonization of pathogenic microorganisms as well as solubilization of immune complexes, needed in a normal immune response. For this reason, therapeutic strategies that include C5a and its receptor are considered an especially promising approach to complement inhibition. For example, therapy with anti-C5 monoclonal antibody (MoAb) has proven effective in preventing collagen-induced arthritis in mice and in ameliorating established disease. In other studies anti-C5a MoAb improved endothelial dysfunction in cardiopulmonary bypass [125,126,128]. Furthermore, an anti-human-C5 MoAb is in phase II studies in patients with rheumatoid arthritis and in phase I studies in patients with active lupus nephritis [125].

In our own studies anti-C5 MoAb reversed aPL-induced thrombophilia and endothelial cell activation in mice [129]. A complement C5a receptor antagonist peptide: AcPhe [Ornithine-Pro-D-cyclohexylananine-Trp-Arg] (C5aR-AP) has specific anti-C5a effects in rats and has been shown to have potent *in vivo* anti-inflammatory activities in murine models of endotoxic shock, renal ischemia-reperfusion injury and the Arthus reaction [130-133]. C5aR-

AP has also been demonstrated to inhibit effects of C5a on human polymorphonuclear cells and human vascular ECs [132]. Coversin (rEV576), a C5 inhibitor isolated from the saliva of the tick *Ornithodoros moubata*, was recently shown by our group to significantly inhibit venous thrombosis in the presence of aPL in a mouse model [134]. Coversin has proven to be an effective therapeutic agent in preclinical models of myasthenia gravis, Guillain Barré syndrome, sepsis and asthma and our results indicate a potential therapeutic role for coversin in primary thromboprophylaxis and in preventing the extension of acute venous thrombosis in APS patients [134-136].

9. Thrombotic and non-thrombotic effects of aPL antibodies associated with pregnancy morbidity

Given the prothrombotic nature of the disease, impairment of maternal-fetal blood exchange as a result of thrombus formation in the uteroplacental vasculature was thought to be the main pathogenic mechanism underlying pregnancy morbidity in APS [137]. However, there is evidence to suggest that placental thrombosis is only partially responsible for APS pregnancy morbidity. Despite placental thrombosis and infarction being demonstrated in some APS patients with first and second trimester abortions, histological evidence of thrombosis in the uteroplacental circulation cannot be demonstrated in the majority of placentas from APS patients [138-140]. IgG fractions from LA positive APS patients can however induce a procoagulant phenotype with significant increases in thromboxane synthesis in placental explants from normal human pregnancies [141]. Interestingly, Rand *et al* have reported significantly lower levels of annexin A5, an important anticoagulant during pregnancy, covering the intervillous surfaces of placentas in women with aPL when compared to controls [142]. *In vitro* studies have also demonstrated displacement of annexin A5 from trophoblast and endothelial cell monolayers by aPL antibodies while murine studies have demonstrated the necessity of this protein in maintaining placental integrity [43,143]. Anti-annexin A5 antibodies have been reported in APS patients at frequencies up to 30% and several studies have demonstrated the association of these Abs with recurrent fetal loss in APS patients [44,144].

There is growing evidence for a direct effect of aPL antibodies on trophoblasts supported by the fact that β_2GPI and anionic PLs are normally expressed on the outer leaflet of trophoblast membranes under physiological conditions due to high levels of tissue remodeling, also explaining the placental tropism of aPL antibodies[145]. *In vitro* studies utilizing murine and human monoclonal aPL antibodies and polyclonal IgG antibodies from APS patients have demonstrated β_2GPI dependent binding of these antibodies to trophoblast monolayers [146,147]. These aPL antibodies have been shown to react with syncytiotrophoblast and to prevent intertrophoblast fusion, trophoblast invasiveness and hCG secretion [146,148,149]. Finely tuned regulation of cell surface adhesion and signaling molecule expression, activation of matrix metalloproteinases (MMPs), angiogenesis and spiral artery transformation characterizes the complex and dynamic process that is placentation [150]. Induction by aPL antibodies of abnormal trophoblast expression of particular integrins and cadherins potentially affecting decidual invasion has been demonstrated *in vitro* [151]. Anti-β_2GPI monoclonal antibodies can inhibit the proliferation of a human choriocarcinoma cell line and extravillous trophoblast differentiation *in vitro* and endometrial biopsy samples from APS patients with recurrent abortions have shown

impaired endometrial differentiation [148,152]. Inhibition of endometrial angiogenesis by aPL antibodies has been demonstrated in a recent study assessing *in vitro* human endometrial endothelial cell (HEEC) angiogenesis and *in vivo* angiogenesis in a murine model. Human polyclonal IgG aPL antibodies were shown to significantly decrease the number and total length of tubule formation, VEGF and MMP production and NF-κB DNA binding activity in HEEC and to reduce new vessel formation in inoculated mice [153].

There is extensive evidence for an inflammatory component to the pathology associated with pregnancy morbidity in APS patients. Polyclonal and monoclonal β_2GPI dependent aPL antibodies can bind stromal decidua cell monolayers and induce a pro-inflammatory phenotype characterized by increased ICAM-1 expression and TNFα secretion [154]. Diminished expression of the complement regulatory protein DAF (decay accelerating factor) has been demonstrated in endometrial biopsy samples from APS patients with recurrent pregnancy loss underscoring the importance of complement activation [152]. Pregnant mice inoculated with human IgG aPL antibodies from APS patients with obstetric APS manifestations had increased rates of fetal resorption, fetal growth retardation and extensive placental damage characterized by recruitment of neutrophils, upregulated TF and TNF-α secretion, decidual focal necrosis and apoptosis, loss of fetal membrane elements and complement deposition [96]. These effects were abrogated in mice given inhibitors of classical and alternative complement pathways and in mice that were C3, C4 or factor B deficient pointing to the involvement of all complement pathways in aPL mediated pregnancy morbidity [155,111]. Additional murine studies have demonstrated the importance of C5a-C5a receptor interactions, especially on neutrophils and monocytes, in inducing TF production, oxidative damage, diminished VEGF levels and subsequent placental hypoperfusion and injury, fetal growth restriction and resorption [111,156,157].

10. Current and potential new treatments for APS-associated clinical manifestations

The cornerstone of treatment for APS remains conventional anticoagulation. Patients who have experienced a venous thromboembolic event (VTE) and are positive for an aPL antibody should be treated with an initial course of unfractionated heparin (UFH), low molecular weight heparin (LMWH) or pentasaccharide followed by warfarin [158]. The initial target intensity of oral anticoagulation is a goal international normalized ratio (INR) of 2.0-3.0 [158]. Patients that suffer an arterial thrombotic event on this regimen should be treated with higher intensity anticoagulation with a goal INR of >3.0 or standard intensity oral anticoagulation (INR 2.0-3.0) in combination with low dose aspirin (LDA). Patients who are intolerant of oral anticoagulation (e.g. inability to achieve and maintain target INR, excessive anticoagulation or adverse effect of warfarin) may be treated with long-term LMWH [159]. Patients who are pregnant should not be treated with warfarin therapy due to potential teratogenicity; rather use of LDA in combination with heparin (Rai, 1998) or LMWH [160,161].

The therapeutic management as well as the prevention of recurrent thrombosis in APS has been focused on utilizing anti-thrombotic medications. Recurrences, despite seemingly adequate treatment, have been reported and the use of oral anticoagulation at a relatively high INR for a long period of time has been associated with a high risk of bleeding, with the need for frequent monitoring and patient compliance with diet and lifestyle to optimize the

therapy. Moreover, still debated is the approach to patients with aPL antibodies without a previous thrombotic event. Some physicians would recommend prophylaxis with low dose aspirin although there are no evidence-based data supporting that low dose aspirin alone is sufficient for primary thrombosis prophylaxis [162]. It is well known that aPL antibodies might be persistently present in the serum of APS patients for long periods of time, but thrombotic events do occur only occasionally. It has been suggested that aPL antibodies (*first hit*) increase the thrombophilic threshold (i.e. induce a prothrombotic/ proinflammmatory phenotype in endothelial cells), but that clotting takes place only in the presence of a *second hit* or triggering event (i.e.: an infection, a surgical procedure, use of estrogens, prolonged immobilization, etc) [89]. Current treatments of thrombosis in APS are directed towards modulating the final event or "second hit". Treatments that modulate early effects of aPL antibodies on target cells – i.e. monocytes or endothelial cells - (*first hit*) would be more beneficial and potentially less harmful than current treatments.

Barriers to the development of new drugs for APS include the multifactorial nature of thrombosis, controversies about the strength of association between aPL antibodies and thrombotic events, and the fact that the mechanisms of aPL-induced thrombosis are not well understood. In the long-term management of APS patients, controlled studies with warfarin alternatives and the new anticoagulant agents (such as oral direct and indirect thrombin inhibitors) as well as newer therapeutic agents are vital. However, it is possible that the current "antithrombotic" approach to aPL-positive patients will be replaced by an "immunomodulatory" approach in the future as our understanding of the mechanisms of aPL-mediated thrombosis grows. Understanding the molecular mechanisms triggered by aPL antibodies and identifying biomarkers released as a consequence of cellular activation may help to design new ways to treat clinical manifestations in APS. Based on data from mechanistic *in vitro* and *in vivo* studies, new targeted treatments may be proposed including: specific inhibition of tissue factor, blocking binding of the aPL antibodies to target cells (i.e.: platelets, endothelial cells, monocytes, trophoblasts, etc), using p38 MAPK inhibitors, NF-κB inhibitors or GPIIb/IIIa inhibitors, abrogating the activation of complement, or targeting cytokines such as IL-6 and TNF-α. Most of these have been discussed in other sections of this chapter (Table 3). Clinical trials are needed to demonstrate whether any of those new therapies are safe and efficacious in APS patients [98,100-103,163-167].

Statins, hydroxychloroquine and rituximab in APS.

Three FDA approved drugs – statins, hydroxychloroquine (HCQ) and rituximab – are also being considered as possible new treatments for APS-associated clinical manifestations based on the effects of these drugs on *in vitro* and *in vivo* animal studies.

a. Statins in APS.

Statins are potent inhibitors of cholesterol synthesis in the mevalonate pathway. In the general population, clinical trials of statin therapy have demonstrated beneficial effects in primary and secondary prevention of coronary heart disease as well as ischemic stroke [168]. However, their beneficial effects are only partially explained by their ability to lower cholesterol levels. Pleiotropic effects of statins have been reported, which include decreasing the expression of CAMs in monocytes and affecting leukocyte /endothelial interactions, down-regulating inflammatory cytokines in endothelial cells or increasing fibrinolytic activity 169-171].

Target or Medication	Supportive Evidence Based on In Vitro and/or Animal Studies	Supportive Evidence Based on aPL(+) Human Studies
Tissue Factor (TF)	Dilazep inhibitis aPL-induced TF upregulation in monocytes and endothelial cells (EC)	No
Nuclear Factor (NF)-κB	NF-κB inhibition decreases aPL-induced upregulation of TF in EC and aPL-enhanced thrombosis in mice	No
P38 Mitogen Activated Protein Kinase (MAPK)	P38MAPK inhibition decreases aPL-induced upregulation of TF in EC, platelet activation, and aPL-enhanced thrombosis in mice	No
Platelet Glycoprotein (GP) Receptors	GP receptor antagonists decrease the aPL-mediated enhancement of platelet activation and abrogate aPL-induced thrombus formation in mice	No
Hydroxychlor-oquine (HCQ)	HCQ decreases aPL-induced platelet activation, inhibits aPL-mediated thrombosis in mice, and protects aPL-induced displacement of Annexin A5 from phospholipids bilayers	Possibly protective against thrombosis in lupus patients A trial will be started Spring 2012
Statins	Statins reverse aPL-induced endothelial cell activation and TF upregulation, and abrogates enhanced thrombus formation in mice	Statins decrease pro-inflammatory and pro-thrombotic markers (pilot data, small number of patients)
β_2GPI and/or anti-β_2GPI binding to Target Cells	Peptides that mimic domains of β_2GPI or β_2GPI receptor blockers (e.g., anti-annexin A2, anti-TLR4, aPOER2 antagonists) inhibit aPL-induced EC activation and/or aPL-mediated thrombosis in mice.	No
Complement	Anti-C5 monoclonal antibody decreases aPL-mediated thrombus formation in mice; anti- C5aRA peptide inhibits aPL-mediated thrombosis and TF expression in mice	No
B Cells	B-cell activating factor (BAFF) blockage can prevent the disease onset in antiphospholipid syndrome mouse model	Rituximab is effective for non-criteria aPL manifestations based on the anecdotal reports

Table 3. Potential Immunomodulatory Approaches in Antiphospholipid-Antibody (aPL) Positive Patients.

There have been numerous publications recently on the benefit of statins in the medical community following the recent results from the JUPITER study, in which patients with normal LDL levels of less than 130 mg/dL and elevated C-reactive protein (CRP), levels greater than 2.0 mg/dL, receiving rosuvastatin 20 mg daily experienced significant reduction in cardiovascular events, non-fatal myocardial infarction, and non-fatal stroke [172].

Studies have suggested that fluvastatin has beneficial effects on aPL-mediated pathogenic effects. First, one study showed that fluvastatin prevented the expression of CAMs and IL-6 in EC treated with aPL antibodies [173]. Subsequently, Ferrara *et al* showed that the thrombogenic and pro-inflammatory effects of aPL antibodies *in vivo* could be abrogated in mice fed with fluvastatin for 15 days [97] and this effect was independent of the cholesterol lowering effects of the drug. The same group of investigators then showed that fluvastatin inhibited the effects of aPL antibodies on tissue factor expression on endothelial cells *in vitro* at doses utilized to reduce cholesterol levels in patients [174]. Furthermore, Martinez *et al*. demonstrated that rosuvastatin decreases VCAM-1 expression by human umbilical vein endothelial cells (HUVEC) exposed to APS serum in an *in vitro* model [175].

Subsequently, Murthy *et al* examined whether proinflammatory/prothrombotic markers are elevated in patients with aPL antibodies and whether treatment with fluvastatin has an effect on those. (Clinical Trials.gov Identifier: NCT00674297). The preliminary analysis of this ongoing pilot study showed that fluvastatin 40 mg daily for 3 months significantly reduced the pro-inflammatory and prothrombotic biomarkers IL-6, IL-1β, sTF, sICAM-1, sVCAM-1 and E-selectin in persistently aPL-positive patients with or without SLE [176]. Furthemore, utilizing proteomic analysis, Cuadrado *et al* have shown that inflammatory proteins can be reversed following one month of treatment with fluvastatin [177].

In summary, although statins have been used in primary and secondary cardiovascular disease prevention, no conclusive data exist for thrombosis prevention in aPL-positive patients. Based on data available, it is conceivable that statins may be beneficial in reversing upregulation of TF, CAMs and inflammatory cytokines in EC and monocytes. Upon successful completion of clinical trials, in theory, statins might even replace warfarin and antiplatelet agents in prevention of recurrent arterial and venous thrombosis, thus eliminating the risk of hemorrhagic complications associated with warfarin and enabling better life style in these patients. Statins may also serve as an alternative treatment in APS patients who experience thrombosis despite adequate anticoagulation with warfarin or with antiplatelet agents, or in those with thrombocytopenia in whom warfarin is contraindicated. Finally, statins would be an appealing prophylactic therapy in patients with high levels of aPL antibodies and without a history of thrombosis. Statins are teratogenic and therefore their use in pregnancy is contraindicated. Side effects must be closely monitored, including elevated liver function tests and potential hyperglycemia and diabetes mellitus. The use of statins in the management of patient with APS needs to be further delineated in well-designed mechanistic and clinical studies.

b. Hydroxychloroquine

Hydroxychloroquine (HCQ) is an antimalarial drug, although the precise mechanism of its anti-inflammatory action is not known. In addition to its anti-inflammatory effects, there are immunomodulatory effects of HCQ that include increasing the pH of intracellular vacuoles

and interfering with antigen processing and inhibiting T-cell-receptor-induced and B-cell-receptor-induced calcium signaling [178,179]. HCQ also has antithrombotic effects by inhibiting platelet aggregation and arachidonic acid release from stimulated platelets [180]. In the general population, HCQ has been historically used as a prophylactic agent against deep vein thrombosis and pulmonary embolism after hip surgeries [181].

HCQ is now considered an essential therapeutic choice in the management of lupus. HCQ has been shown to decrease the probability of lupus flares, the accrual of damage, and possibly protect SLE patients from vascular and thrombotic events [181-183]. Furthermore, HCQ may facilitate the response to other agents in SLE patients with renal involvement. More recently, chloroquine and HCQ have been shown to improve survival in a cohort of 232 SLE patients after adjusting for patient characteristics and disease activity [184]. It has been recently suggested that HCQ may affect TLR9 activation and IFN-α production and this drug is now considered an essential therapeutic choice in the management of lupus.

In aPL-injected mice, HCQ decreases the thrombus size and the aPL-enhanced thrombus formation in a dose-dependent manner [95]. Furthermore, HCQ inhibits the aPL-induced expression of platelet GPIIb/IIIa receptor (platelet activation) in a dose-dependent fashion [115]. Recently, using 3D atomic microscopy force height images, Rand *et al* showed that HCQ also reverses the binding of aPL-β_2GPI complexes to phospholipids bilayers [185,186]. In SLE patients, those receiving HCQ experienced fewer thrombotic events and in the Baltimore Lupus Cohort, investigators showed a decreased risk of arterial thrombosis [187]. Other investigators demonstrated that HCQ decreases the risk of thrombosis in patients with SLE (OR 0.67). In a Cox multiple failure time analysis, HCQ was shown to protect against thrombosis and increase survival in patients with SLE. In a cross-sectional study in which Erkan *et al* compared 77 APS patients with previous vascular events (65% had no other systemic autoimmune diseases) to 56 asymptomatic (no history of thrombosis or fetal loss) aPL-positive patients (18% had no other systemic autoimmune diseases), logistic regression analysis suggested that HCQ protects against thrombosis in asymptomatic aPL-positive individuals [188]. In summary, although there is experimental and clinical evidence that HCQ might decrease the incidence of thrombosis in patients with SLE, both detailed mechanistic and controlled studies are needed to determine the effectiveness of HCQ for primary and secondary thrombosis prevention in patients with APS. At this time, even though there are insufficient data to recommend HCQ for primary and secondary prevention, it might be reasonable to add HCQ to anticoagulation agents in APS patients who develop recurrent thrombosis despite optimum anticoagulation.

Multiple studies have shown reduction in thrombotic events in SLE patients receiving HCQ [189,182]. However, despite some studies showing a sharp contrast to this demonstrated protective effect, it appears reasonable that HCQ can be used a second line agent, in addition to anti-coagulation, in patients with APS and thrombus. As well, before starting therapy, it is important to screen for macular toxicity with visual field and fundoscopic examination every six to twelve months.

A prospective blind-placebo control clinical trial of persistently aPL-positive individuals will soon be started by an international multicenter collaborative effort under the auspices of APS ACTION (Antiphospholipid Syndrome: Alliance for Clinical Trials and International Networking). The primary objective of this trial is to determine the efficacy of HCQ therapy

in primary thrombo-prophylaxis in persistently aPL-positive APS patients with no history of thrombosis or any other systemic autoimmune disease.

c. Rituximab.

Recently, rituximab has been shown to be a good therapeutic agent for life-threatening CAPS in a small number of patients [190-192]. Rituximab has been successfully used in case reports of patients with aPL and auto-immune mediated thrombocytopenia and hemolytic anemia. A systematic review of the off-label use of rituximab in APS revealed the higher rate of therapeutic response in patients with APS (92%) [193] and an increasing number of similar case reports clearly indicates the need for clinical trials to evaluate the effect of rituximab in the treatment of resistant APS. Currently Erkan *et al* are conducting a RITAPS open-label Phase II trial using Rituximab to study patients who are aPL positive and resistant to conventional anticoagulation (Clinical trials.gov Identifier: NCT00537290). In preliminary results reported at a recent annual meeting of the American College of Rheumatology in 2011, the investigators reported that although a net decrease of aPL antibody titers was not seen in patients given rituximab, the drug appeared to have an effect on improving thrombocytopenia, and skin ulcers accompanied by an overall decrease in CD19+ B cells. [194].

11. Concluding remarks

Since the mid-1980s, aPL antibodies and their associated clinical manifestations have attracted great interest among clinicians and investigators. Indeed, the attention directed to aPL often exceeds that for other autoantibodies within the field of autoimmunity; even in systemic lupus erythematosus, which is characterized by a multitude of specificities, the interest in this serological system remains high.

A significant amount of knowledge has been gained in the last 20 years with respect to etio-pathogenesis of this complex disease. In addition, progress has been accomplished on standardization of "criteria" aPL tests as well as new emerging tests and methodologies that may help to improve the diagnosis of APS. Recently, the improved understanding of the intracellular and molecular mechanisms activated during aPL-induced thrombosis has enabled investigators to propose new and possibly more effective - with less harmful side effects - treatments of APS-related clinical manifestations. Clinical trials for these new treatments are urgently needed (some already have been started) to translate bench research into new therapies for affected patients.

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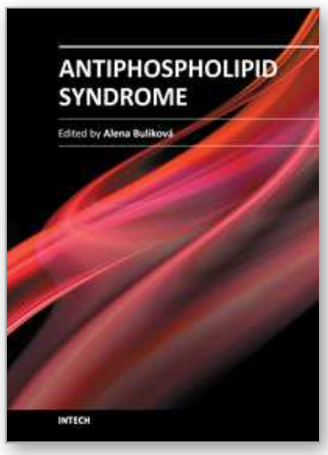

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The antiphospholipid syndrome has been described for the first time by Graham Hughes in 1983 as a condition connected with thromboses or foetal losses and antiphospholipid antibodies presence. Form that time there has been a great progress in knowledge, including antiphospholipid antibodies characterisation, their probable and also possible action, clinical manifestations, laboratory detection and treatment possibilities . This book provides a wide spectrum of clinical manifestations through Chapters written by well known researchers and clinicians with a great practical experience in management of diagnostics or treatment of antiphospholipid antibodies' presence.

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