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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- β 2 glycoprotein I (anti- β 2GPI) 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 heterogeneous 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 β 2glycoprotein 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 thrombophlebitis 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_2 GPI 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_2 GPI antibody	Positive for IgG or IgM isotype (in titer > the 99 th percentile) on two or more occasions, at least 12 weeks apart measured by standardized ELISA

Miyakis et al J Thromb Haemost 2006; 4; 295-306.

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_2 GPI 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_2 GPI 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_2 GPI 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].

<p>Subgroup 1 International Consensus Guidelines on assay performance of aCL and anti-β_2GPI assay.</p>	<p>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. <i>Arthritis Rheum</i> 2011 Sep 27. doi: 10.1002/art.33349)</p>
<p>Subgroup 1 Guidelines on use of calibrator for aCL/ anti-β_2GPI assays and selection and preparation of reference material</p>	<p>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. <i>Clin Chim Acta</i>. 2011 Oct 15. [Epub ahead of print])</p>
<p>Subgroup 2 Review of the Updated ISTH SCC guidelines on the use of Lupus anti-coagulant (LAC) for diagnosis.</p>	<p>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.</p>
<p>Subgroup 3 Role of aPL as “risk factors”.</p>	<p>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</p>

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.

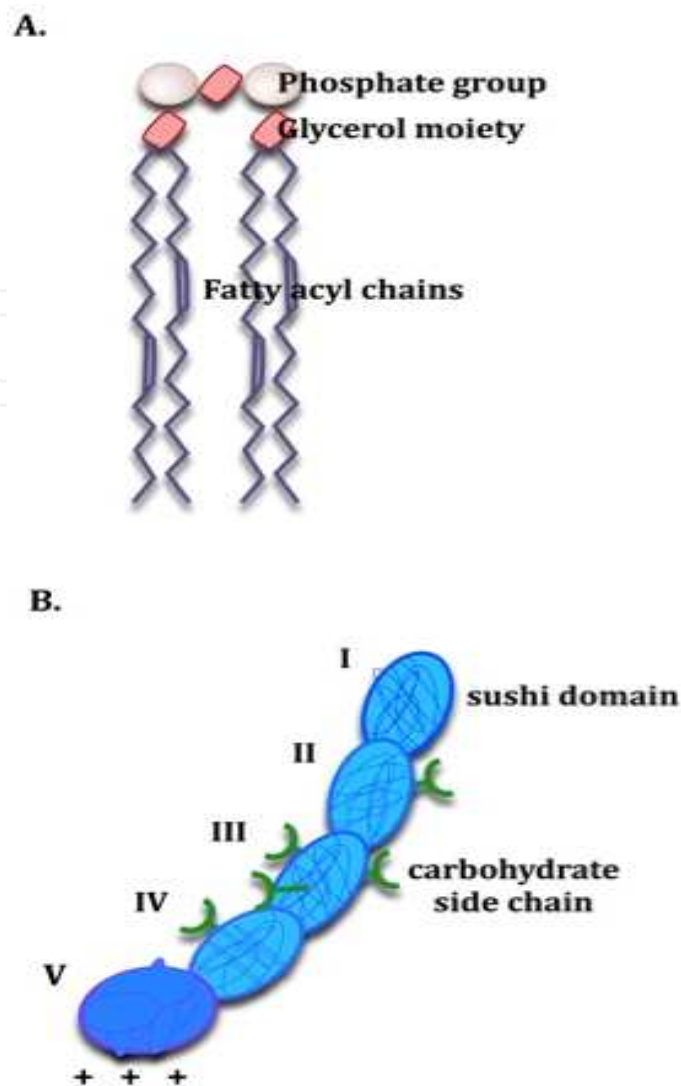
Several autoantibodies have been demonstrated to bind directly to negatively charged phospholipids other than CL (individually or as a phospholipid mixture) or to other proteins in the coagulation cascade (i.e., prothrombin and/or phosphatidylserine-prothrombin complexes); antibodies can also interfere 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_2 GPI, which is now recognized as the main antigenic target of pathogenic aPL [2]. Indeed, β_2 GPI 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_2 GPI consists of five contiguous domains (Figure 1b), the first proposed to be the binding site for pathogenic anti- β_2 GPI 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_2 GPI 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_2 GPI 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_2 GPI Abs complexed with β_2 GPI 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 vitro* studies have demonstrated the role of annexin A2 (AnnA2), in association with Toll-like receptor 4 (TLR4) and/or apolipoprotein ER2'



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Fig. 1. Schematic representation of Cardiolipin and of β_2 Glycoprotein I.

A. Cardiolipin structure contains 2 negatively charged phosphate head groups, 3 glycerol moieties and 4 fatty acyl chains

B. β_2 -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 I α (GPIb α) 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_2 GPI /anti- β_2 GPI 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_2 GPI rather than pure phospholipids [47]. This led researchers to believe that perhaps *in vivo* binding of foreign PL-binding proteins resembling β_2 GPI 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_2 GPI known to be a major PL-binding site of the molecule, and demonstrated the peptide's ability to induce pathogenic aPL and anti- β_2 GPI antibody production in mice [48]. A monoclonal antibody with aPL and anti- β_2 GPI 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_2 GPI 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_2 GPI 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_2 GPI Ab [53]. Following immunization with *Haemophilus influenzae*, *Neisseria gonorrhoeae* or tetanus toxoid; high titers of antibodies of anti-peptide (TLRVYK) and anti- β_2 GPI 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_2 GPI, inducing conformational changes, exposing hidden epitopes and in so doing then enable production of anti- β_2 GPI 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_2 GPI 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 \times BXSB F1 (W/B F1) male mice that displayed spontaneous production of IgG aCL antibodies which exhibit co-factor (β_2 GPI) 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 germline 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_2 GPI 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 occurrence 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_2 GPI, 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_2 GPI 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?

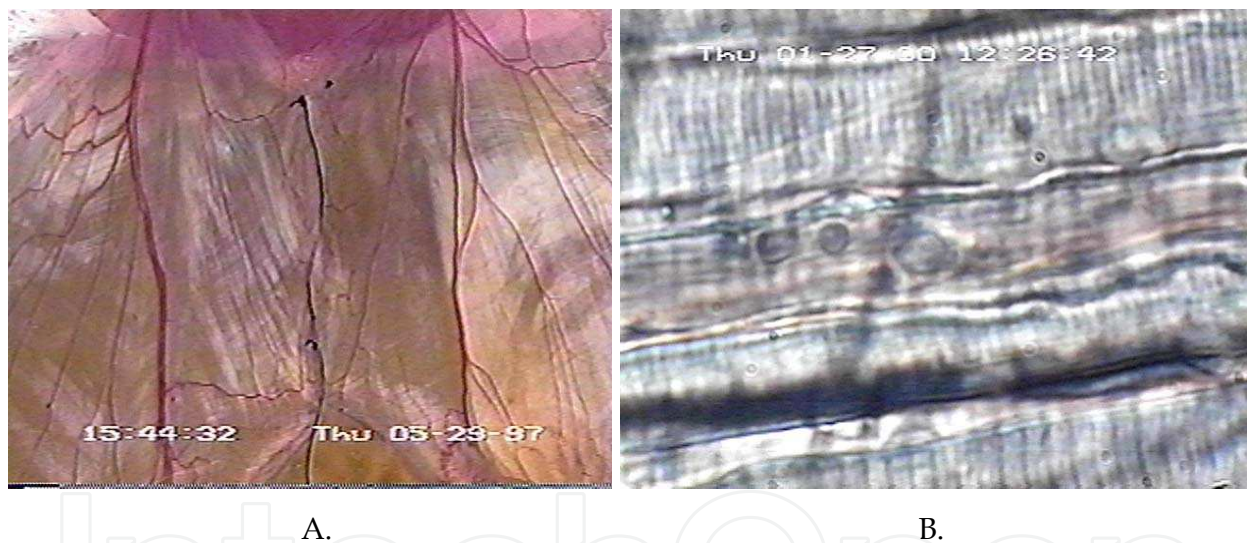
a. Animal Models of Thrombosis and Endothelial Cell Activation

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_2 GPI or human aPL antibodies also had thrombogenic properties *in vivo* [87]. Furthermore, murine monoclonal aPL and a monoclonal antibody obtained by immunization with the phospholipid-binding domain of β_2 GPI, 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_2 GPI in hamsters and rats respectively [88,89]. More recently, Arad and colleagues showed in an animal model that affinity purified anti- β_2 GPI 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].



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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_2 GPI/anti- β_2 GPI 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-Pedrerá *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 (TNF α) 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 from 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-cyclohexylalanine-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_2 GPI 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_2 GPI 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_2 GPI 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- κ B 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_2 GPI dependent aPL antibodies can bind stromal decidua cell monolayers and induce a pro-inflammatory phenotype characterized by increased ICAM-1 expression and TNF α 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/proinflammatory 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- κ B 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 inhibits aPL-induced TF upregulation in monocytes and endothelial cells (EC)	No
Nuclear Factor (NF)-κB	NF- κ B 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
Hydroxychloroquine (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_2 GPI or β_2 GPI 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-1 β , sTF, sICAM-1, sVCAM-1 and E-selectin in persistently aPL-positive patients with or without SLE [176]. Furthermore, 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_2 GPI 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.

12. References

- [1] Miyakis S, Lockshin MD, Atsumi I et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4:295-306
- [2] McNeil HP, Simpson RJ, Cherterman CN et al. Antiphospholipid antibodies are directed against a complex antigen that includes lipid binding inhibitor of coagulation: β 2 glycoprotein I (apolipoprotein H). *Proc Natl Acad Sci USA* 1990; 87:4120-4
- [3] Roubey RA. Antiphospholipid syndrome: antibodies and antigens. *Curr Opin Hematol* 2000; 7(5):316-320.

- [4] Ginsburg KS, Liang MH, Newcomer L et al. Anticardiolipin antibodies and the risk for ischemic stroke and venous thrombosis. *Ann Intern Med* 1992; 117:997-1002
- [5] McClain MT, Arbuckle MR, Heinlen LD et al. The prevalence, onset and clinical significance of antiphospholipid antibodies prior to diagnosis of systemic lupus erythematosus. *Arthritis Rheum* 2004;50:1226-32
- [6] Petri M. Update on anti-phospholipid antibodies in SLE: the Hopkins' Lupus Cohort. *Lupus* 2010; 19:419-23
- [7] Asherson RA. Multiorgan failure and antiphospholipid antibodies: the catastrophic antiphospholipid (Asherson's) syndrome. *Immunobiology* 2005; 210: 727-33
- [8] Sailer T, Zaglami C, Kurz C et al. Anti-beta(2)-glycoprotein-I antibodies are associated with pregnancy loss in women with the lupus anticoagulant. *Thromb Haemost* 2006; 5:796-801.
- [9] Moore JE, Mohr CF. Biologically false positive serologic tests for syphilis; type, incidence, and cause. *J Am Med Assoc* 1952; 150: 467-473
- [10] Conley CL, Hartmann RC. A hemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus. *J Clin Invest* 1952; 31:621-622
- [11] Feinstein DI, Rapaport SI. Acquired inhibitors of blood coagulation. *Prog Hemostas Thromb* 1972;1:75-95.
- [12] Bowie EJ, Thompson JH Jr, Pascuzzi CA, Owen CA Jr. Thrombosis in Systemic Lupus Erythematosus despite circulating anticoagulants. *J Lab Clin Med.* 1963; 62:416-30.
- [13] Thiagarajan P, Shapiro SS, De Marco L. Monoclonal immunoglobulin M lambda coagulation inhibitor with phospholipid specificity. Mechanism of lupus anticoagulant. *J Clin Invest* 1980;66:397-405.
- [14] Harris EN. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis. *Lancet* 1983; 2: 1211-1214.
- [15] Boey ML, Colaco CB, Gharavi AE, Elkon KB, Loizou S, Hughes GR. Thrombosis in SLE: striking associations with the presence of circulating "lupus anticoagulant" *Br Med J.* 1983; 287: 1021-3.
- [16] Elias M, Eldor A. Thromboembolism in patients with the "lupus" like circulating anticoagulant. *Arch Int Med.* 1984; 144: 510-515
- [17] Harris EN, Pierangeli SS. Primary, secondary, and catastrophic antiphospholipid syndrome: what's in a name? *Semin Throm Haemost* 2008; 34:219-226
- [18] 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: 1428-1432
- [19] Cervera R, Tektonidou MG, Espinosa G, et al. "Task Force on Catastrophic Antiphospholipid Syndrome (APS) and Non-Criteria APS Manifestations (I): catastrophic APS, APS nephropathy and heart valve lesions." *Lupus.* 2011; 20: 165-173.
- [20] Bucciarelli S, Espinosa G, Cervera R, et al. "Mortality in the Catastrophic Antiphospholipid Syndrome: Causes of Death and Prognostic Factors in a Series of 250 Patients." *Arthritis Rheum.* 2006; 54(8): 2568-2576.
- [21] Ciubotaru C, Esfahani F, Benedict RHB, Wild LM, Baer AN. "Chorea and Rapidly Progressive Subcortical Dementia in Antiphospholipid Syndrome." *J Clin Rheumatol.* 2002; 8: 332-339.

- [22] Cervera R, Tektonidou MG, Espinosa G, et al. "Task Force on Catastrophic Antiphospholipid Syndrome (APS) and Non-Criteria APS Manifestations (II): thrombocytopenia and skin manifestations." *Lupus*. 2011; 20: 174-181.
- [23] Pierangeli SS, Harris EN. A quarter of a century in anticardiolipin antibody testing and attempted standardization has led us to here, which is?. *Semin Thromb Haemost* 2008;334:313-328.
- [24] Pierangeli S, Groot P, Dlott J, et al. "Criteria" aPL tests: Report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, TX. April 2010. *Lupus* 2011; 20: 182-190.
- [25] Lakos G, Favaloro EJ, Harris EN, Meroni PL, Tincani A, Wong RC et al. International consensus guidelines on anticardiolipin and anti-beta(2) glycoprotein I testing: A report from the APL task force at the 13(th) international congress on antiphospholipid antibodies. *Arthritis Rheum* 2011 Sep 27. doi: 10.1002/art.33349. [Epub ahead of print]
- [26] Pierangeli S, Favaloro EJ, Lakos G, Meroni PL, Tincani A, Wong RC, Harris EN. Standards and reference materials for the anticardiolipin and anti- β (2)glycoprotein I assays: A report of recommendations from the APL Task Force at the 13th International Congress on Antiphospholipid Antibodies. *Clin Chim Acta*. 2011 Oct 15. [Epub ahead of print]
- [27] Bertolaccini ML, Amengual O, Atsumi T, et al. "Non-criteria" aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies. *Lupus* 2011; 20: 191-205.
- [28] Permpikul P, Rao LV, Rapaport SI. Functional and binding studies of the roles of prothrombin and beta 2-glycoprotein I in the expression of lupus anticoagulant activity. *Blood*. 1994 May 15;83(10):2878-92.
- [29] de Laat B, Pengo V, Pabinger I, Musial J, Voskuyl AE, Bultink IE et al. The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study. *J Thromb Haemost*. 2009 Nov;7(11):1767-73
- [30] Martin SJ, Reutelingsperger CP, McGahon AJ, Rader JA, van Schie RC, LaFace DM, Green DR. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J Exp Med*. 1995 Nov 1;182(5):1545-56.
- [31] Kobayashi K, Kishi M, Atsumi T, Bertolaccini ML, Makino H, Sakairi N et al. Circulating oxidized LDL forms complexes with beta2-glycoprotein I: implication as an atherogenic autoantigen. *J Lipid Res*. 2003 Apr;44(4):716-26.
- [32] Lopez LR, Simpson DF, Hurley BL, Matsuura E. OxLDL/beta2GPI complexes and autoantibodies in patients with systemic lupus erythematosus, systemic sclerosis, and antiphospholipid syndrome: pathogenic implications for vascular involvement. *Ann N Y Acad Sci*. 2005 Jun;1051:313-22.
- [33] Cuadrado MJ, Buendía P, Velasco F et al. Vascular endothelial growth factor expression in monocytes from patients with primary antiphospholipid syndrome. *J Thromb Haemost*. 2006 Nov;4(11):2461-9

- [34] Pierangeli SS, Colden-Stanfield M, Liu X et al. Antiphospholipid antibodies from antiphospholipid syndrome patients activate endothelial cells in vitro and in vivo. *Circulation*. 1999 Apr 20;99(15):1997-2002
- [35] Chamley LW, Duncalf AM, Mitchell MD, Johnson PM. Action of anticardiolipin and antibodies to beta2-glycoprotein-I on trophoblast proliferation as a mechanism for fetal death. *Lancet*. 1998 Sep 26;352(9133):1037-8
- [36] Shi T, Giannakopoulos B, Yan X et al. Anti-beta2-glycoprotein I antibodies in complex with beta2-glycoprotein I can activate platelets in a dysregulated manner via glycoprotein Ib-IX-V. *Arthritis Rheum*. 2006 Aug;54(8):2558-67
- [37] Pierangeli SS, Vega-Ostertag ME, Raschi E et al. Toll-like receptor and antiphospholipid mediated thrombosis: in vivo studies. *Ann Rheum Dis*. 2007 Oct;66(10):1327-33
- [38] Sorice M, Longo A, Capozzi A et al. Anti-beta2-glycoprotein I antibodies induce monocyte release of tumor necrosis factor alpha and tissue factor by signal transduction pathways involving lipid rafts. *Arthritis Rheum*. 2007 Aug;56(8):2687-97
- [39] Romay-Penabad Z, Aguilar-Valenzuela R, Urbanus RT, Derksen H et al. Apolipoprotein E receptor 2' is involved in the thrombotic complications in a murine model of the antiphospholipid syndrome. *Blood*. 2011 Jan 27;117(4):1408-14.
- [40] Urbanus RT, Pennings MT, Derksen RH, de Groot PG. Platelet activation by dimeric beta2-glycoprotein I requires signaling via both glycoprotein I α and apolipoprotein E receptor 2'. *J Thromb Haemost*. 2008 Aug;6(8):1405-12
- [41] Sikara MP, Routsias JG, Samiotaki M et al. β 2 Glycoprotein I (β 2GPI) binds platelet factor 4 (PF4): implications for the pathogenesis of antiphospholipid syndrome. *Blood*. 2010 Jan 21;115(3):713-23
- [42] Chen PP, Giles I. Antibodies to serine proteases in the antiphospholipid syndrome. *Curr Rheumatol Rep*. 2010 Feb;12(1):45-52.
- [43] Rand JH, Wu XX, Guller S et al. Antiphospholipid immunoglobulin G antibodies reduce annexin-V levels on syncytiotrophoblast apical membranes and in culture media of placental villi. *Am J Obstet Gynecol*. 1997 Oct;177(4):918-23
- [44] Nojima J, Kuratsune H, Suehisa E et al. Association between the prevalence of antibodies to beta(2)-glycoprotein I, prothrombin, protein C, protein S, and annexin V in patients with systemic lupus erythematosus and thrombotic and thrombocytopenic complications. *Clin Chem*. 2001 Jun;47(6):1008-15
- [45] Ortona E, Capozzi A, Colasanti T, Conti F, Alessandri C, Longo A et al. Vimentin/cardioliipin complex as a new antigenic target of the antiphospholipid syndrome. *Blood*. 2010 Oct 21;116(16):2960-7.
- [46] Jiang H, Chess L. How the immune system achieves self-nonsel self discrimination during adaptive immunity. *Adv Immunol*. 2009;102:95-133.
- [47] Gharavi AE, Sammaritano LR, Wen J, Elkon KB. Induction of antiphospholipid autoantibodies by immunization with beta 2 glycoprotein I (apolipoprotein H). *J Clin Invest*. 1992 Sep;90(3):1105-9
- [48] Gharavi AE, Pierangeli SS, Colden-Stanfield M et al. GDKV-induced antiphospholipid antibodies enhance thrombosis and activate endothelial cells in vivo and in vitro. *J Immunol*. 1999 Sep 1;163(5):2922-7

- [49] Gharavi AE, Pierangeli SS, Gharavi EE et al. Thrombogenic properties of antiphospholipid antibodies do not depend on their binding to beta2 glycoprotein 1 (beta2GP1) alone. *Lupus*. 1998;7(5):341-6.
- [50] Gharavi EE, Chaimovich H, Cucurull E et al. Induction of antiphospholipid antibodies by immunization with synthetic viral and bacterial peptides. *Lupus*. 1999;8(6):449-55
- [51] Gharavi AE, Pierangeli SS, Espinola RG et al. Antiphospholipid antibodies induced in mice by immunization with a cytomegalovirus-derived peptide cause thrombosis and activation of endothelial cells in vivo. *Arthritis Rheum*. 2002 Feb;46(2):545-52
- [52] Gharavi AE, Vega-Ostertag M, Espinola RG et al. Intrauterine fetal death in mice caused by cytomegalovirus-derived peptide induced aPL antibodies. *Lupus*. 2004;13(1):17-23
- [53] Blank M, Krause I, Fridkin M et al. Bacterial induction of autoantibodies to beta2-glycoprotein-I accounts for the infectious etiology of antiphospholipid syndrome. *J Clin Invest*. 2002 Mar;109(6):797-804
- [54] Levy Y, Almog O, Gorshtein A, Shoenfeld Y. The environment and antiphospholipid syndrome. *Lupus*. 2006;15(11):784-90
- [55] Uthman IW, Gharavi AE. Viral infections and antiphospholipid antibodies. *Semin Arthritis Rheum*. 2002 Feb;31(4):256-63.
- [56] Sène D, Piette JC, Cacoub P. Antiphospholipid antibodies, antiphospholipid syndrome and viral infections. *Rev Med Interne*. 2009 Feb;30(2):135-41
- [57] VAN Os GM, Meijers JC, Agar C, Seron MV, Marquart JA, Akesson P et al. Induction of anti- $\beta(2)$ -glycoprotein I autoantibodies in mice by protein H of *Streptococcus pyogenes*. *J Thromb Haemost*. 2011 Dec;9(12):2447-56.
- [58] Rauch J, Dieudé M, Subang R, Levine JS. The dual role of innate immunity in the antiphospholipid syndrome. *Lupus*. 2010 Apr;19(4):347-53.
- [59] Aguilar-Valenzuela R, Nickerson K, Romay-Penabad Z, Shlomchik MJ, Vargas G, Shilagard T, Pierangeli S. Involvement of TLR7 and TLR9 in the production of antiphospholipid antibodies. *Arthritis Rheum* 2011; 63(10):s281 (abstract 723)
- [60] Hashimoto Y, Kawamura M, Ichikawa K et al. Anticardiolipin antibodies in NZW x BXS B F1 mice. A model of antiphospholipid syndrome. *J Immunol*. 1992 Aug 1;149(3):1063-8
- [61] Kita Y, Sumida T, Iwamoto I, Yoshida S, Koike T. V gene analysis of anti-cardiolipin antibodies from (NZW x BXS B) F1 mice. *Immunology*. 1994 Jul;82(3):494-501.
- [62] Ida A, Hirose S, Hamano Y et al. Multigenic control of lupus-associated antiphospholipid syndrome in a model of (NZW x BXS B) F1 mice. *Eur J Immunol*. 1998 Sep;28(9):2694-703
- [63] Papalardo E, Romay-Penabad Z, Christadoss P, Pierangeli S. Induction of pathogenic antiphospholipid antibodies in vivo are dependent on expression of MHC-II genes. *Lupus* 2010; 19:496 (abstract)
- [64] . Exner T, Barber S, Kronenberg H, Rickard KA. Familial association of the lupus anticoagulant. *Br J Haematol*. 1980 May;45(1):89-96
- [65] Jolidon RM, Knecht H, Humair L, de Torrente A. Different clinical presentations of a lupus anticoagulant in the same family. *Klin Wochenschr*. 1991 May 24;69(8):340-4

- [66] Matthey F, Walshe K, Mackie IJ, Machin SJ. Familial occurrence of the antiphospholipid syndrome. *J Clin Pathol*. 1989 May;42(5):495-7
- [67] Dagenais P, Urowitz MB, Gladman DD, Norman CS. A family study of the antiphospholipid syndrome associated with other autoimmune diseases. *J Rheumatol*. 1992 Sep;19(9):1393-6
- [68] Rouget JP, Goudemand J, Montreuil G et al. Lupus anticoagulant: a familial observation. *Lancet*. 1982 Jul 10;2(8289):105.
- [69] Mackie IJ, Colaco CB, Machin SJ. Familial lupus anticoagulants. *Br J Haematol*. 1987 Nov;67(3):359-63
- [70] May KP, West SG, Moulds J, Kotzin BL. Different manifestations of the antiphospholipid antibody syndrome in a family with systemic lupus erythematosus. *Arthritis Rheum*. 1993 Apr;36(4):528-33
- [71] Arnett FC, Olsen ML, Anderson KL, Reveille JD. Molecular analysis of major histocompatibility complex alleles associated with the lupus anticoagulant. *J Clin Invest*. 1991 May;87(5):1490-5
- [72] Asherson RA, Doherty DG, Vergani D et al. Major histocompatibility complex associations with primary antiphospholipid syndrome. *Arthritis Rheum*. 1992 Jan;35(1):124-5
- [73] Caliz R, Atsumi T, Kondeatis E et al. HLA class II gene polymorphisms in antiphospholipid syndrome: haplotype analysis in 83 Caucasoid patients. *Rheumatology (Oxford)*. 2001 Jan;40(1):31-6
- [74] Hashimoto H, Yamanaka K, Tokano Y et al. HLA-DRB1 alleles and beta 2 glycoprotein I-dependent anticardiolipin antibodies in Japanese patients with systemic lupus erythematosus. *Clin Exp Rheumatol*. 1998 Jul-Aug;16(4):423-7
- [75] Wilson WA, Scopelitis E, Michalski JP et al. Familial anticardiolipin antibodies and C4 deficiency genotypes that coexist with MHC DQB1 risk factors. *J Rheumatol*. 1995 Feb;22(2):227-35
- [76] Petri M, Watson R, Winkelstein JA, McLean RH. Clinical expression of systemic lupus erythematosus in patients with C4A deficiency. *Medicine (Baltimore)*. 1993 Jul;72(4):236-44
- [77] Bertolaccini ML, Atsumi T, Caliz AR et al. Association of antiphosphatidylserine/prothrombin autoantibodies with HLA class II genes. *Arthritis Rheum*. 2000 Mar;43(3):683-8
- [78] Vargas-Alarcon G, Granados J, Bekker C et al. Association of HLA-DR5 (possibly DRB1*1201) with the primary antiphospholipid syndrome in Mexican patients. *Arthritis Rheum*. 1995 Sep;38(9):1340-1
- [79] Galeazzi M, Sebastiani GD, Tincani A et al. HLA class II alleles associations of anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus. *Lupus*. 2000;9(1):47-55
- [80] Hirose N, Williams R, Alberts AR et al. A role for the polymorphism at position 247 of the beta2-glycoprotein I gene in the generation of anti-beta2-glycoprotein I antibodies in the antiphospholipid syndrome. *Arthritis Rheum*. 1999 Aug;42(8):1655-61

- [81] Chopra N, Koren S, Greer WL et al. Factor V Leiden, prothrombin gene mutation, and thrombosis risk in patients with antiphospholipid antibodies. *J Rheumatol*. 2002 Aug;29(8):1683-8
- [82] Pierangeli SS, Barker JH, Stikovac D, Ackerman D, Anderson G, Barquinero J, Acland R, Harris EN. Effect of human IgG antiphospholipid antibodies on an in vivo thrombosis model in mice. *Thromb. Haemostas*. 1994; 71(5): 670-4.
- [83] Stockmans F, Deckmyn H, Gruwez J, Vermylen J, Acland R. Continuous quantitative monitoring of mural, platelet-dependent, thrombus kinetics in the crushed rat femoral vein. *Thromb Haemost*. 1991 Apr 8;65(4):425-31.
- [84] Barker JH, Gu JM, Anderson GL, O'Shaughnessy M, Pierangeli S, Johnson P, Galletti G, Acland RD. The effects of heparin and dietary fish oil on embolic events and the microcirculation downstream from a small artery repair. *Plast. Reconstr. Surg* . 1993; 91: 335-342.
- [85] Pierangeli SS, Liu X, Barker JH, Anderson G, Harris EN. Induction of thrombosis in a mouse model by IgG, IgM and IgA immunoglobulins from patients with the Antiphospholipid Syndrome. *Thrombosis Haemost* . 1995; 74(5): 1361-1367.
- [86] Olee T, Pierangeli SS, Handley HH, Novotny W, En J, Harris EN, Woods L, Chen PP. Generation and characterization of Monoclonal IgG Anticardiolipin Antibodies from a Patient with the Antiphospholipid Syndrome. *Proc Nat Acad Sci. (USA)*. 1996; 93: 8606-8611.
- [87] Pierangeli SS, Liu, XW, Anderson G, Barker JH, Harris EN. Thrombogenic properties of murine anti-cardiolipin antibodies induced by β_2 glycoprotein 1 and human IgG antiphospholipid antibodies. *Circulation*. 1996; 94:1746-1751.
- [88] Jankowski M, Vreys I, Wittevrongel C, Boon D, Vermylen J, Hoylaerts MF, Arnout J. Thrombogenicity of beta 2-glycoprotein I-dependent antiphospholipid antibodies in a photochemically induced thrombosis model in the hamster. *Blood*. 2003 Jan 1;101(1):157-62. Epub 2002 Sep 5.
- [89] Fischetti F, Durigutto P, Pellis V, Debeus A, Macor P, Bulla R, Bossi F, Ziller F, Sblattero D, Meroni P, Tedesco F. Thrombus formation induced by antibodies to beta2-glycoprotein I is complement dependent and requires a priming factor. *Blood*. 2005 Oct 1;106(7):2340-6. Epub 2005 Jun 14.
- [90] Arad A, Proulle V, Furie RA, Furie BC, Furie B. β_2 -Glycoprotein-1 autoantibodies from patients with antiphospholipid syndrome are sufficient to potentiate arterial thrombus formation in a mouse model. *Blood*. 2011 Mar 24;117(12):3453-9. Epub 2011 Jan 18.
- [91] Pierangeli SS, Espinola RG, Liu X, Harris EN. Thrombogenic effects of antiphospholipid (aPL) antibodies are mediated by intercellular cell adhesion molecule-1(ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and P-selectin. *Circulation Res* 2001; 88: 245-250.
- [92] Pierangeli SS, Liu Xiaowei, Espinola R, Olee T, Min Zhi, Harris EN and Chen PP. Functional analysis of patient-derived IgG monoclonal anticardiolipin antibodies using in vivo thrombosis and in vivo microcirculation Models. *Thrombosis and Haemostasis* 2000; 84:388-395.
- [93] Pierangeli SS, Espinola RG, Liu X, Harris EN, Salmon J. Identification of an Fc γ receptor independent mechanism by which intravenous immunoglobulin (IVIg)

- ameliorates antiphospholipid antibody-induced thrombogenic phenotype. *Arthritis Rheum* 2001;44: 876-883 (2001)
- [94] Vega-Ostertag ME, Liu X, Henderson V, Pierangeli SS. A peptide that mimics the Vth Region of β_2 glycoprotein I reverses antiphospholipid-mediated thrombosis in mice. *Lupus*. 2006; 15: 358-365.
- [95] Edwards M, Pierangeli SS, Liu Xwei, Barker JH, Anderson GH, Harris EN. Hydroxychloroquine Reverses Thrombogenic Properties of Antiphospholipid Antibodies in Mice. *Circulation* . 1997; 96(12):4380-4384.
- [96] Holers VM, Girardi G, Mo L, Guthridge JM, Molina H, Pierangeli SS, Espinola R, Liu X, Mao D, Vialpando CG, Salmon JE. Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *J Exp Med* 2002; 195(2): 211-220.
- [97] Ferrara DE, Liu X, Espinola RG, Meroni PL, Abujhalaf I, Harris EN, Pierangeli SS. Inhibition of the thrombogenic and inflammatory properties of antiphospholipid antibodies by fluvastatin in an in vivo animal model. *Arthritis Rheum*. 2003; 48(11): 3272-3279.
- [98] Pierangeli SS, Girardi G, Vega-Ostertag ME, Liu X, Espinola RG, Salmon JE. Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia. *Arthritis Rheum* 2005; 52: 2120-2124.
- [99] Vega-Ostertag ME, Liu PP, Henderson V, Chen PP, Pierangeli SS. A human monoclonal anti-prothrombin antibody is thrombogenic in vivo and upregulates expression of tissue factor and E-selectin on endothelial cells. *Br. J. Haematology*. 2006; 135: 214-219.
- [100] Vega-Ostertag ME, Ferrara DE, Romay-Penabad Z, Liu X, Taylor WR, Colden-Stanfield M, Pierangeli SS. Role of p38 mitogen-activated protein kinase in antiphospholipid antibody-mediated thrombosis and endothelial cell activation. *J Thromb Haemost* 2007; 5: 1828-1834.
- [101] Montiel-Manzano G, Romay-Penabad Z, Papalardo de Martinez E, Meillon-Garcia LA, Garcia-Latorre E, Reyes-Maldonado, Pierangeli SS. In vivo effects of an inhibitor of nuclear factor-kappa B on thrombogenic properties of antiphospholipid antibodies. *Ann N Y Acad Sci* 2007; 1108: 540-553.
- [102] Romay-Penabad Z, Liu XX, Montiel-Manzano G, Papalardo de Martinez E, Pierangeli SS. C5a receptor-deficient mice are protected from thrombophilia and endothelial cell activation induced by some antiphospholipid antibodies. *Ann N Y Acad Sci* 2007; 1108: 554-566.
- [103] Ioannou Y, Romay-Penabad Z, Pericleous C, Giles I, Papalardo E, Vargas G, Shilagard T, Latchman DS, Isenberg D, Rahman A, Pierangeli S. A novel concept for the in vivo inhibition of antiphospholipid antibody induced vascular thrombosis through the use of the antigenic target peptide domain I of β_2 glycoprotein I. *J. Thromb Haemost* 2009; 7:833-842.
- [104] Giles I, Pericleous C, Liu X, Ehsanullaj J, Clarke L, Brogan P, Newton-West M, Swerlick R, Lambrianides N, Chen P, Latchman D, Isenberg D, Pierangeli SS, Rahman A. Thrombin binding predicts the effects of sequence changes in a human monoclonal antiphospholipid antibodies on its in vivo biological actions. *J Immunol* 2009; 182:4836-4843.

- [105] Romay-Penabad Z, Montiel-Manzano G, Shilagard T, Vargas G, Deora A, Wang M, Garcia-Latorre E, Reyes-Maldonado E, Hajjar KA, Pierangeli S. Annexin A2 is involved in antiphospholipid antibody-mediated pathogenic effects in vitro and in vivo. *Blood* 2009; 114:3074-3083.
- [106] Aron AL, Cuellar ML, Brey RL, Mckeown S, Espinoza LR, Shoenfeld Y, Gharavi AE. Early onset of autoimmunity in MRL/++ mice following immunization with beta 2 glycoprotein I. *Clin Exp Immunol*. 1995 July; 101(1): 78-81
- [107] Branch DW, Dudley DJ, Mitchell MD, Creighton KA, Abbott TM, Hammond EH, Daynes RA. Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss. *Am J Obstet. Gynecol*. 1990; 163:210-216.
- [108] Bakimer R, Fishman P, Blank M et al. Induction of primary antiphospholipid syndrome in mice by immunization with a human monoclonal anticardiolipin antibody (H-3). *J Clin Invest* 1992; 89: 1558-63.
- [109] Gharavi AE, Vega-Ostertag ME, Espinola RG, et al. Intrauterine fetal death in mice caused by cytomegalovirus-derived peptide induced by aPL antibodies. *Lupus* 2004; 13:17-23.
- [110] Fishman P, Falach-Vaknine E, Zigelman, R Bakimer R, Sredni B, Djaldetti M, Shoenfeld Y. Prevention of fetal loss in experimental antiphospholipid syndrome by in vivo administration of recombinant interleukin-3. *J Clin Invest*. 1993 April; 91(4): 1834-1837.
- [111] Girardi G, Berman J, Redecha P et al. Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. *J Clin Invest*. 2003 Dec;112(11):1644-54
- [112] Girardi G, Redecha P, Salmon JE. Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. *Nat Med* 2004; 10: 1222-1226.
- [113] Khamashta MA, Harris EN, Gharavi AE et al. Immune mediated mechanism for thrombosis: antiphospholipid antibody binding to platelet membranes. *Ann Rheum Dis*. 1988 Oct;47(10):849-54
- [114] Lutters BC, Derksen RH, Tekelenburg WL et al. Dimers of beta 2-glycoprotein I increase platelet deposition to collagen via interaction with phospholipids and the apolipoprotein E receptor 2'. *J Biol Chem*. 2003 Sep 5;278(36):33831-8
- [115] Espinola RG, Pierangeli SS, Gharavi AE, Harris EN. Hydroxychloroquine reverses platelet activation induced by human IgG antiphospholipid antibodies. *Thromb Haemost*. 2002 Mar;87(3):518-22
- [116] Pierangeli SS, Vega-Ostertag M, Harris EN. Intracellular signaling triggered by antiphospholipid antibodies in platelets and endothelial cells: a pathway to targeted therapies. *Thromb Res*. 2004;114(5-6):467-76
- [117] Meroni PL, Raschi E, Camera M et al. Endothelial activation by aPL: a potential pathogenetic mechanism for the clinical manifestations of the syndrome. *J Autoimmun*. 2000 Sep;15(2):237-40
- [118] Simantov R, Lo SK, Gharavi A et al. Antiphospholipid antibodies activate vascular endothelial cells. *Lupus*. 1996 Oct;5(5):440-1

- [119] Amengual O, Atsumi T, Khamashta MA, Hughes GR. The role of the tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid syndrome. *Thromb Haemost.* 1998 Feb;79(2):276-81
- [120] Kornberg A, Blank M, Kaufman S, Shoenfeld Y. Induction of tissue factor-like activity in monocytes by anti-cardiolipin antibodies. *J Immunol.* 1994 Aug 1;153(3):1328-32
- [121] Reverter JC, Tàssies D, Font J et al. Effects of human monoclonal anticardiolipin antibodies on platelet function and on tissue factor expression on monocytes. *Arthritis Rheum.* 1998 Aug;41(8):1420-7
- [122] Alijotas-Reig J, Palacio-Garcia C, Vilardell-Tarres M. Circulating microparticles, lupus anticoagulant and recurrent miscarriages. *Eur J Obstet Gynecol Reprod Biol.* 2009 Jul;145(1):22-6
- [123] López-Pedraza Ch, Buendía P, Cuadrado MJ, et al; Antiphospholipid antibodies from antiphospholipid syndrome patients induce monocyte expression through the simultaneous activation of both NFκB/Rel proteins via p38 MAPK pathway, and the MEK1/ERK pathway. *Arthritis Rheum.* 2006;54:301-311.
- [124] Jajoria P, Murthy V, Papalardo E, Romay-Penabad Z, Gleason C, Pierangeli SS. Statins for the treatment of antiphospholipid syndrome? *Ann N Y Acad Sci.* 2009 Sep;1173:736-45
- [125] Tesser J, et al. Safety and efficacy of the humanized anti-C5a antibody h5G1.1 in patients with rheumatoid arthritis. *Arthritis Rheum* 2001; 44: s274 (abstract).
- [126] Wang Y, Rollins SA, Madri JA, Matis LA. Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. *Proc Natl Acad Sci USA.* 1995; 92: 8955-8959.
- [127] Quigg RJ. Use of complement inhibitors in tissue injury. *Trends Mol Med* 2002; 8:430-436.
- [128] Tofukuji M, Stahl GL, Agah A, Metais C, Simons M, Sellke FW. 1998. Anti-C5 monoclonal antibody reduces cardiopulmonary bypass and cardioplegia-induced coronary endothelial dysfunction. *J Thorac. Cardiovasc Durg* 116:1060-1068.
- [129] Arumugam TV, Shiels IA, Strachan AJ, Abbenante G, Fairlie DP, Taylor SM. A small molecule C5a receptor antagonist protects kidneys from ischemia/reperfusion injury in rats. *Kidney Int* 2003; 63:134-142.
- [130] Strachan AJ, Woodruff TM, Haaima G, Fairlie DP, Taylor SM. A new small molecule C5a-receptor antagonist inhibits the reverse-passive Arthus reaction and endotoxic shock in rats. *J Immunol* 2000; 164: 6560-6565.
- [131] Patel KLH, Farrar CA, Hargreaves EG, Sacks SH, Zhou W. Complement activation regulates the capacity of proximal tubular epithelial cell to stimulate alloreactive T cell response. *J Am Soc Nephrol* 2004; 15:2414-2422.
- [132] Finch AM, Wong AK, Paczkowski NJ, Wadi SK, Craik DJ, Fairlie DP, Taylor SM. Low molecular-weight peptidic and cyclic antagonist of the receptor for the complement factor C5a. *J Med Chem* 1999; 42:1965-1974.
- [133] Mastellos D, Papadimitriou JC, Franchini S, Pangiotis AT, Lambris JD. A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration. *J Immunol* 2001; 166: 2479-2486.

- [134] Carrera-Marin AL, Romay-Penabad Z, Machin S, Cohen H, Pierangeli S. C5 inhibitor rEV576 ameliorates in vivo effects of antiphospholipid antibodies. *Arthritis Rheum* 2011; 63(10):s5 (abstract 12)
- [135] Soltys J, Kusner LL, Young A, Richmonds C, Hatala D et al. Novel complement inhibitor limits severity of experimentally myasthenia gravis. *Ann Neurol*. 2009 Jan;65(1):67-75.
- [136] Halstead SK, Humphreys PD, Zitman FM, Hamer J, Plomp JJ, Willison HJ. C5 inhibitor rEV576 protects against neural injury in an in vitro mouse model of Miller Fisher syndrome. *J Peripher Nerv Syst*. 2008 Sep;13(3):228-35.
- [137] De Wolf F, Carreras LO, Moerman P et al. Decidual vasculopathy and extensive placental infarction in a patient with repeated thromboembolic accidents, recurrent fetal loss, and a lupus anticoagulant. *Am J Obstet Gynecol*. 1982 Apr 1;142(7):829-34
- [138] Hanly JG, Gladman DD, Rose TH et al. Lupus pregnancy. A prospective study of placental changes. *Arthritis Rheum*. 1988 Mar;31(3):358-66
- [139] Nayar R, Lage JM. Placental changes in a first trimester missed abortion in maternal systemic lupus erythematosus with antiphospholipid syndrome; a case report and review of the literature. *Hum Pathol*. 1996 Feb;27(2):201-6
- [140] Out HJ, Kooijman CD, Bruinse HW, Derksen RH. Histopathological findings in placentae from patients with intra-uterine fetal death and anti-phospholipid antibodies. *Eur J Obstet Gynecol Reprod Biol*. 1991 Oct 8;41(3):179-86
- [141] Peaceman AM, Rehnberg KA. The effect of immunoglobulin G fractions from patients with lupus anticoagulant on placental prostacyclin and thromboxane production. *Am J Obstet Gynecol*. 1993 Dec;169(6):1403-6
- [142] Rand JH, Wu XX, Guller S et al. Reduction of annexin-V (placental anticoagulant protein-I) on placental villi of women with antiphospholipid antibodies and recurrent spontaneous abortion. *Am J Obstet Gynecol*. 1994 Dec;171(6):1566-72
- [143] Wang X, Campos B, Kaetzel MA, Dedman JR. Annexin V is critical in the maintenance of murine placental integrity. *Am J Obstet Gynecol*. 1999 Apr;180(4):1008-16
- [144] Donohoe S, Kingdom JC, Mackie IJ et al. Ontogeny of beta 2 glycoprotein I and annexin V in villous placenta of normal and antiphospholipid syndrome pregnancies. *Thromb Haemost*. 2000 Jul;84(1):32-8
- [145] McIntyre JA. Immune recognition at the maternal-fetal interface: overview. *Am J Reprod Immunol*. 1992 Oct-Dec;28(3-4):127-31
- [146] Di Simone N, Meroni PL, de Papa N et al. Antiphospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered beta2-glycoprotein I. *Arthritis Rheum*. 2000 Jan;43(1):140-50
- [147] Katsuragawa H, Kanzaki H, Inoue T et al. Monoclonal antibody against phosphatidylserine inhibits in vitro human trophoblastic hormone production and invasion. *Biol Reprod*. 1997 Jan;56(1):50-8
- [148] Adler RR, Ng AK, Rote NS. Monoclonal antiphosphatidylserine antibody inhibits intercellular fusion of the choriocarcinoma line, JAR. *Biol Reprod*. 1995 Oct;53(4):905-10
- [149] Rote NS, Vogt E, DeVere G et al. The role of placental trophoblast in the pathophysiology of the antiphospholipid antibody syndrome. *Am J Reprod Immunol*. 1998 Feb;39(2):125-36

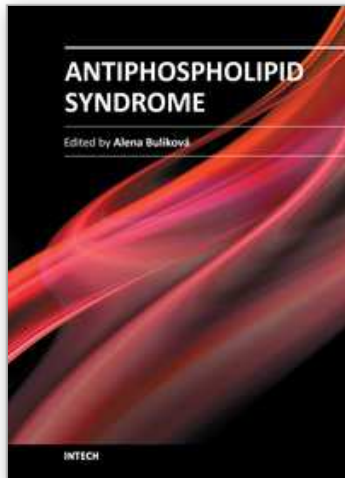
- [150] Castellucci M, De Matteis R, Meisser A et al. Leptin modulates extracellular matrix molecules and metalloproteinases: possible implications for trophoblast invasion. *Mol Hum Reprod*. 2000 Oct;6(10):951-8
- [151] Di Simone N, Castellani R, Caliendo D, Caruso A. Antiphospholipid antibodies regulate the expression of trophoblast cell adhesion molecules. *Fertil Steril*. 2002 Apr;77(4):805-11
- [152] Francis J, Rai R, Sebire NJ et al. Impaired expression of endometrial differentiation markers and complement regulatory proteins in patients with recurrent pregnancy loss associated with antiphospholipid syndrome. *Mol Hum Reprod*. 2006 Jul;12(7):435-42
- [153] Di Simone N, Di Nicuolo F, D'Ippolito S et al. Antiphospholipid antibodies affect human endometrial angiogenesis. *Biol Reprod*. 2010 Aug 1;83(2):212-9
- [154] Borghi MO, Raschi E, Scurati S et al. Effects of a toll-like receptor antagonist and anti-annexin A2 antibodies on binding and activation of decidual cells by anti- β 2glycoprotein I antibodies. *Clin Exp Rheumatol* 2007. 2:35
- [155] Quigg RJ, Kozono Y, Berthiaume D et al. Blockade of antibody-induced glomerulonephritis with Crry-Ig, a soluble murine complement inhibitor. *J Immunol*. 1998 May 1;160(9):4553-60
- [156] 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 Oct 1;110(7):2423-31
- [157] Girardi G, Yarilin D, Thurman JM et al. Complement activation induces dysregulation of angiogenic factors and causes fetal rejection and growth restriction. *J Exp Med*. 2006 Sep 4;203(9):2165-75
- [158] Ruiz-Irastora G, Hunt BJ, Khamashta MA. "A systematic review of secondary thromboprophylaxis in patients with antiphospholipid antibodies." *Arthritis Rheum*. 2007; 57: 1487-95
- [159] Vargas-Hitos JA, Ateka-Barrutia O, Sangle S, Khamashta MA. "Efficacy and safety of long-term low molecular weight heparin in patients with antiphospholipid syndrome." *Ann Rheum Dis*. 2011; 70: 1652-1654.
- [160] Rai R, Cohen H, Dave M, Regan L. "Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies)". *BMJ*. 1997; 314: 253-257.
- [161] Alalaf S. "Bemiparin versus low dose aspirin for management of recurrent early pregnancy loss due to antiphospholipid antibody syndrome." *Arch Gynecol Obstet*. 2011. ePub ahead of print.
- [162] Erkan D, Harrison M, Levy R, et al. Aspirin for primary thrombosis prevention in the antiphospholipid syndrome: a randomized, double-blind, placebo-controlled trial in asymptomatic antiphospholipid antibody-positive individuals. *Arthritis Rheum* 2007; 56: 2382-2391.
- [163] Zhou H, Wolberg AS, Roubey AS. Characterization of monocyte tissue factor activity induced by IgG antiphospholipid antibodies and inhibition by dilazep. *Blood* 2004; 15: 2353-2358

- [164] Ostertag MV, Liu X, Henderson V, Pierangeli SS. A peptide that mimics the Vth region of beta-2-glycoprotein I reverses antiphospholipid-mediated thrombosis in mice. *Lupus* 2006;15:358-65.
- [165] Blank M, Shoenfeld Y, Cabilly S, Heldman Y, Fridkin M, Katchalski-Katzir E. of experimental antiphospholipid syndrome and endothelial cell activation by synthetic peptides. *Proc Natl Acad Sci U S A* 1999;96:5164-8.
- [166] Pierangeli SS, Blank M, Liu X, et al. A peptide that shares similarity with bacterial antigens reverses thrombogenic properties of antiphospholipid antibodies in vivo. *J Autoimmun* 2004;22:217-25.
- [167] Carrera-Marin AL, Romay-Penabad Z, Qu HC, et al. A C5a receptor antagonist ameliorates in vivo effects of antiphospholipid antibodies. *Arthritis Rheum* 2009; 60; s767 (abstract).
- [168] Halcox JPJ, Deanfield JE. Beyond the laboratory. Clinical implications for statin pleiotropy. *Circulation* 2004; 109 (suppl II); 42-48
- [169] Colli S, Eligini S, Lalli M, et al. Statins inhibit tissue factor in cultured human macrophages. A novel mechanism of protection against atherothrombosis. *Arterioscler Thromb Vasc Biol* 1997; 17: 265-272.
- [170] Aikawa M, Rabkin E, Sugiyama S, et al. An HMG-CoA inhibitor, cerivastatin, suppresses growth of macrophages expressing matrix metalloproteinases and tissue factor in vivo and in vitro, *Circulation* 2001; 103:276-283.
- [171] Baetta R, Camera M, Comparato C, et al. Tremoli E. Fluvastatin reduces tissue factor expression and macrophage accumulation in carotid lesions of cholesterol-fed rabbits in absence of lipid lowering. *Arterioscler Thromb Vasc Biol* 2002; 22:692-698.
- [172] Ridker PM, Danielson E, Fonseca FA, et al: JUPITER Trial Study Group. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. *Lancet* 2009; 373: 1175-1182.
- [173] Meroni PL, Raschi E, Testoni C, et al. Statins prevent endothelial cell activation induced by antiphospholipid (anti- β_2 glycoprotein I) antibodies: effect on the proadhesive and proinflammatory phenotype. *Arthritis Rheum* 2001; 44:2870-2878.
- [174] Ferrara DE, Swerlick R, Casper K, et al. Fluvastatin inhibits upregulation of tissue factor expression by antiphospholipid antibodies on endothelial cells. *J Thromb Haemost* 2004; 2: 1558-1563.
- [175] Martinez-Martinez LA, Amigo MC, Orozco A, et al. Effect of rosuvastatin on VCAM-1 expression by HIVEC exposed to APS serum in an in vitro model. *Clin Exp Rheumatol* 2007; 25:18-19.
- [176] Murthy V, Erkan D, Jajoria P, Willis R, Vega J, Barilaro B, Basra G, Hsu E, Martinez-Martinez LA, Jatwani S, Papalardo E, Gonzalez EB, Sunkureddi PR, Pierangeli S. Effects of Fluvastatin on Pro-Inflammatory and Pro-Thrombotic Markers in Antiphospholipid Antibody (aPL)-Positive Patients: Preliminary Results from an Open-Label Prospective Pilot Study. *Arthritis Rheum* 2011; 63(10):s283 (abstract 726)

- [177] Cuadrado MJ, Lopez-Pedrerera C, Aguirre A, et al. Changes Operated in Protein Pattern of Monocytes from Patients with Antiphospholipid Syndrome Treated with Statins. *Arthritis Rheum* 2007; 56: S782 (abstract)
- [178] Lombard-Platlet S, Bertolino P, Deng H, Gerlier D, Rabourdin-Combe C. Inhibition by chloroquine of the class II major histocompatibility complex-restricted presentation of endogenous antigens varies according to the cellular origin of the antigen-presenting cells, the nature of the T-cell epitope, and the responding T cell. *Immunology* 1993; 80: 566–573.
- [179] Goldman FD, Gilman AL, Hollenback C, et al. Hydroxychloroquine inhibits calcium signals in T cells: a new mechanism to explain its immunomodulatory properties. *Blood* 2000; 95: 3460–3466.
- [180] Jancinova V, Nosal R, Petrikova M. On the inhibitory effect of chloroquine on blood platelet aggregation. *Thromb Res* 1994; 74: 495–504.
- [181] Johnson R, Charnley J. Hydroxychloroquine in prophylaxis of pulmonary embolism following hip arthroplasty. *Clin Orthop Relat Res* 1979; 144: 174–177.
- [182] Erkan D, Yazici Y, Peterson MG et al. A cross-sectional study of clinical thrombotic risk factors and preventive treatments in antiphospholipid syndrome. *Rheumatology (Oxford)* 2002; 41:9249-929.
- [183] Ho KT, Ahn CW, Alarcon GS, et al. Systemic lupus erythematosus in a multiethnic cohort (LUMINA): XXVIII. Factors predictive of thrombotic events. *Rheumatology (Oxford)* 2005; 44:1303-1307.
- [184] Tektonidou MG, Laskari K, Panagiotakis DB, Moutsopoulos HM. Risk factors for thrombosis and primary thrombosis prevention in patients with systemic lupus erythematosus with or without antiphospholipid antibodies. *Arthritis Rheum* 2009; 61:29-35.
- [185] Rand JH, Wu XX, Quinn AS, et al. Hydroxychloroquine directly reduces the binding of antiphospholipid antibody-beta2-glycoprotein I complexes to phospholipid bilayers. *Blood* 2008; 112: 1687–1695.
- [186] Rand JH, Wu XX, Quinn AS, et al. Hydroxychloroquine protects the annexin A5 anticoagulant shield from disruption by antiphospholipid antibodies: evidence for a novel effect for an old antimalarial drug. *Blood*. 2009 Nov 30. [Epub ahead of print]
- [187] Petri M. Lupus in Baltimore: evidence-based ‘clinical pearls’ from the Hopkins Lupus Cohort *Lupus* 2005; 14: 970-973.
- [188] Kaiser R, Cleveland CM, Criswell LA. Risk and protective factors for thrombosis in systemic lupus erythematosus: results from a large, multi-ethnic cohort. *Ann Rheum Dis Ann Rheum Dis* 2009; 68: 238-241.
- [189] Ruiz-Irastorza G, Egurbide MV, Pijoan JL, et al. Effect of antimalarials on thrombosis and survival in patients with systemic lupus erythematosus. *Lupus* 2006; 15: 577–583.
- [190] Rubenstein E, Arkfeld DG, Metyas S, et al. Rituximab treatment for resistant antiphospholipid syndrome. *J Rheumatol* 2006; 33: 355–357.
- [191] Tenedious F, Erkan D, Lockshin MD. Rituximab in the primary antiphospholipid antibody syndrome. *Arthritis Rheum* 2005; 52: 4078 (abstract).

- [192] Erdozain JG, Ruiz-Irastorza G, Egurbide MV, et al. Sustained response to rituximab of autoimmune hemolytic anemia associated with antiphospholipid syndrome. *Haematologica* 2004;89:ECR34 [abstract].
- [193] Erre GL, Pardini S, Faedda R, et al. Effect of rituximab on clinical and laboratory features of antiphospholipid syndrome: a case report and a review of literature. *Lupus* 2008; 17: 50-55.
- [194] Erkan D, Vega J, Ramon G, Kozora E, Lockshin MD. Rituximab in Antiphospholipid Syndrome (RITAPS)—A Pilot Open-Label Phase II Prospective Trial for Non-Criteria Manifestations of Antiphospholipid Antibodies (aPL). *Arthritis Rheum* 2011; 63(10):s283 (abstract 727)

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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. From 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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