International Immunopharmacology 27 (2015) 200–208

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Immunotherapy in antiphospholipid syndrome

Ch. Lopez-Pedrera [a,*], M.A. Aguirre [a], P. Ruiz-Limon [a], C. Pérez-Sánchez [a], Y. Jimenez-Gomez [a], N. Barbarroja [a], M.J. Cuadrado [b]

[a] Maimonides Institute for Research in Biomedicine of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Cordoba, Córdoba, Spain
[b] Lupus Research Unit, St. Thomas Hospital, London, UK

ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 20 April 2015
Accepted 3 June 2015
Available online 15 June 2015

Keywords:
Antiphospholipid syndrome
Pathogenesis
New therapeutic approaches

ABSTRACT

Antiphospholipid syndrome (APS) is a disorder characterized by the association of arterial or venous thrombosis and/or pregnancy morbidity with the presence of antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant antibodies, and/or anti-β2-glycoprotein I antibodies).
Thrombosis is the major manifestation in patients with aPLs, but the spectrum of symptoms and signs associated with aPLs has broadened considerably, and other manifestations, such as thrombocytopenia, non-thrombotic neurological syndromes, psychiatric manifestations, livedo reticularis, skin ulcers, hemolytic anemia, pulmonary hypertension, cardiac valve abnormality, and atherosclerosis, have also been related to the presence of those antibodies.
Several studies have contributed to uncovering the basis of antiphospholipid antibody pathogenicity, including the targeted cellular components, affected systems, involved receptors, intracellular pathways used, and the effector molecules that are altered in the process.
Therapy for thrombosis traditionally has been based on long-term oral anticoagulation; however, bleeding complications and recurrence despite high-intensity anticoagulation can occur. The currently accepted first-line treatment for obstetric APS (OAPS) is low-dose aspirin plus prophylactic unfractionated or low-molecular-weight heparin (LMWH). However, in approximately 20% of OAPS cases, the final endpoint, i.e. a live birth, cannot be achieved.
Based on all the data obtained in different research studies, new potential therapeutic approaches have been proposed, including the use of new oral anticoagulants, statins, hydroxychloroquine, coenzyme Q10, B-cell depletion, platelet and TF inhibitors, peptide therapy or complement inhibition among others. Current best practice in use of these treatments is discussed.

1. Pathogenic mechanisms in the antiphospholipid syndrome

1.1. Activation of tissue factor and related pro-thrombotic molecules

Many mechanisms have been proposed to explain the thrombotic tendency of patients with APS, but the pathogenesis seems to be multifactorial. Procoagulant cell activation, accompanied by tissue factor (TF) expression and TF pathway upregulation, is one of the key events in the pathophysiology of thrombosis in patients with APS. Previous studies showed elevated plasma levels of soluble TF in APS patients, and thereafter we reported that monocytes isolated from APS patients had high TF expression [1–3]. At the molecular level, the signal transduction mechanisms induced by aPLs have been explored. An ex-vivo study led us to show that aPLs induced TF in monocytes from APS patients by activating – simultaneously and independently – the phosphorylation of mitogen-activated protein kinase (MAPK)/extracellular regulated kinase protein, and the p38 MAPK-dependent nuclear translocation and activation of nuclear factor-κB (NF-kB)/Rel proteins [4]. Similar results have been reported in platelets, monocyte cell lines, and in vivo models of aPL-induced thrombogenicity [5–7]. Parallel studies performed in endothelial cells (ECs) further concluded that: 1) NF-κB plays an essential role in TF activation induced by aPLs [8]; and 2) p38 MAPK phosphorylation and NF-κB activation are involved in the aPL-induced increase in TF transcription, function, and expression; interleukin (IL)-6 and IL-8 upregulation; and inducible nitric oxide synthase expression [9]. Previous reports indicate a close relationship between TF and vascular endothelial growth factor (VEGF), a family of proteins involved in normal vascular development and in relevant pathophysiologic settings, including cancer, wound healing, and inflammation [10]. Precedent studies had reported increased plasma levels of VEGF in APS patients [11]. Then we analyzed the VEGF and FMS-related tyrosine kinase 1 (FLT1) expression levels in monocytes of APS

* Corresponding author at: IMIBIC, Reina Sofia University Hospital, University of Córdoba, Avda. Menéndez Pidal s/n, E-14004 Córdoba, Spain. Tel.: +34 957 736 542; fax: +34 957 010 340.
E-mail addresses: rosario.lopez.exts@juntadeandalucia.es, charylopezpedrera@gmail.com (C. Lopez-Pedrera).

http://dx.doi.org/10.1016/j.intimp.2015.06.006
1567-5769/

patients, the molecular mechanisms involved in their aPL-induced expression, and their association with the elevated TF expression found in these patients [12]. Our data primarily showed that monocytes from APS patients expressed increased levels of both VEGF and FLT1 in comparison with monocytes from healthy donors. Furthermore, in vitro results indicated that this cytokine was produced by monocytes when treated with aPLs, and that the p38 MAPK signaling pathway played an important role. Thus, VEGF might act as a regulatory factor in aPL-mediated monocyte activation and TF expression, thereby contributing to the proinflammatory–prothrombotic phenotype of APS patients. Moreover, the excess of plasmatic thrombin in APS, most likely induced by TF expression, and acting through the activation of protease activated receptors (PARs 1 and 2, also increased in monocytes of APS [13]), might also be related to the elevated VEGF production found in that patients.

The application of proteomic techniques to APS patients' monocytes has led one to identify an altered expression of proteins. This abnormal expression might be directly related to the pathogenic mechanisms of APS. Our group has addressed the question of predicting thrombotic risk in APS patients by using a proteomic approach on purified human monocytes [14]. The proteins identified as more significantly deregulated in the monocytes from patients with APS and thrombosis were annexin A1 (AnxA1), annexin A2 (AnxA2), ubiquitin Nedd8, Rho A protein, protein disulfide isomerase (PDI) and Hsp60. These proteins have been shown to be associated with the induction of a procoagulant state, as well as autoimmune-related responses. In that way, Anx A2 has recently been directly involved in the pathogenesis of APS. It has been demonstrated that binding of β2GPI to human umbilical vein endothelial cells is mediated by Anx A2 [15]. Furthermore, thrombosis and TF upregulation are significantly decreased in Anx A2 deficient mice in vivo. By functioning as a receptor for β2GPI, Anx A2 is a target not only for anti-Anx A2 antibodies but also for anti-β2GPI antibodies, which are direct inductors of TF. These data suggest that Anx A2 might constitute a common receptor for aPL induction of monocyte activation [16]. Protein disulfide isomerase (PDI) has been also demonstrated to be linked to TF on the cell surface when coagulant activity is low and TF-VIIa signaling is enabled. Moreover, PDI expression reduction has been associated with a two-fold increase of TF procoagulant activity [17]. Overexpression of PDI suppresses NF-κB-dependent transcriptional activity [18]. As the aberrant activation of the NF-κB signaling pathway is likely to contribute to the development of APS, the decrease expression of this protein might be related to the constitutive activation of this transcription factor in the APS. In addition, as described below, the PDI has a crucial role in development of the immunogenic form of β2GPI in the setting of APS.

1.2. Atherogenesis

Experimental studies and human observations suggest that APS is associated with atherosclerosis. In fact, innate and adaptive immune responses participate in the pathogenesis of both diseases. Anti-oxLDL, anti-aPL, anti-β2GPI, and anti-HSP antibodies, among others, have been found in patients with APS and atherosclerosis [19]. Endothelial dysfunction, oxidative stress, an increase in cell adhesion molecules, and active platelets are common findings in both diseases. In addition, macrophages, dendritic cells, T-cell activation, and CD40–CD40 ligand interaction are considered pathogenic mechanisms of atherosclerosis and APS [20,21].

Notably, aPL antibodies trigger an inflammatory cascade, and they have been associated with atherosclerosis as well as cerebrovascular and peripheral arterial diseases [22,23]. Moreover, aPL antibodies may cross-react with oxidized low-density lipoproteins (ox-LDLs), and both aPL and anti-ox-LDL antibodies have been implicated in the pathogenesis of atherosclerosis associated with systemic lupus erythematosus (SLE) and APS. It has been shown that aPL antibodies, in particular anti-β2GPI antibodies, can accelerate the influx of ox-LDLs into macrophages [24]. Other autoantibodies, such as anti-high-density lipoproteins (HDLs) and antiapolipoprotein A-I, also have been detected in APS. In addition, macrophages and ECs bind to β2GPI during the atherosclerotic process. In this regard, anticardiolipin (aCL) antibodies can induce monocyte adherence to ECs, which is mediated by adhesion molecules such as ICAM-1, VCAM-1, and E-selectin. Thus, aCL antibodies might promote atherosclerosis by attracting monocytes into the vessel wall. Moreover, a correlation between serum levels of aCL and anti-β2GPI antibodies and the incidence and severity of acute coronary syndrome, myocardial infarction, and stroke have been demonstrated previously [25,26].

Early endothelial dysfunction [27] and increased carotid intima–media thickness also have been observed in APS [28]. Accordingly, in a recent study, within a cohort of 43 APS patients, we found that patients with higher aPL-IgG titers showed a strong association with the development of thrombotic events and also with the increased intimate–media thickness (IMT) of the carotid arteries [29]. The issue of early atherosclerosis development in APS patients has shown controversial data in past years. In our series, the presence of plaques in carotid arteries in a significant number of APS patients was in favor of the evidence of an accelerated atherosclerosis. Our results confirmed previous reports showing greater IMT in APS, related to the titer of aPL-IgG [30–32]. Moreover, our data pointed to the existence of premature atherosclerosis as a clinical feature of thrombotic APS patients, so that in our series, 11 of 12 of the APS patients who presented increased IMT values had suffered at least 1 thrombotic event. In addition, our results agreed with a recent study showing that premature atherosclerosis, as defined by IMT, occurs in thrombotic APS over 30 years [28]. Premature atherosclerosis might be facilitated by the existence of an inflammatory status in APS, which seems not to be coordinated by "classic" cytokines such as TNFα or IL-6 but by other known inflammatory mediators, including VEGF and tPA, as well as various chemokines (IL-8, MCP-1, or MIP-1α) whose main function is to recruit, e.g., neutrophils, monocytes, B cells, and T helper cells to the sites of inflammation [33,34]. Thrombus formation is a key event in the development of the intimae thickening, considered to comprise the early stage of atherosclerosis plaque formation. Many studies have demonstrated that TF is present in atherosclerosis lesions and contributes to atherogenesis [35]. TF mediates the responses that are critical for hemostasis and thrombosis, as well as inflammatory reactions. Thus TF, whose expression is also significantly increased in monocytes of APS patients, together with factors downstream of the coagulation cascade and the PAR2 activation system, would act as an additional multifactorial regulator of atherogenesis.

1.3. Oxidative stress and mitochondrial dysfunction

Various studies have evidenced that oxidative stress is directly involved in the pathophysiology of both APS and SLE. Mitochondrial dysfunction, accompanied with ATP depletion, oxidative stress, abnormal activation, and death signal processing in lupus T cells have been demonstrated previously [36]. In the setting of APS, aCL antibodies seem to play an important role in the oxidative status by inducing nitric oxide (NO) and superoxide production, resulting in enhanced levels of plasma peroxynitrite, a powerful pro-oxidant substance [37]. Titers of aCL antibodies have been found positively correlated to plasma levels of F2-isoprostanes, sensitive markers of in vivo lipid peroxidation, indicating enhanced oxidative stress in APS [38,39]. Functional and structural arterial abnormalities have been associated with lower activity of paraoxonase, an antioxidant enzyme linked to HDLs that prevents LDL oxidation. Moreover, in patients with aPL antibodies, HDL reduced NO bioavailability and showed impaired anti-inflammatory and antioxidant properties [40]. Thus, there is substantial evidence showing oxidative damage to lipids and proteins in APS. In a very recent study [29] we showed an increased production of reactive oxygen species (ROS) by monocytes and neutrophils that disturbs the redox status and in turn may influence the expression of prothrombotic and proinflammatory

molecules. That increase was accompanied by a significant reduction in the capacity of cells to counteract ROS, as demonstrated by the observed reduction in both the intracellular levels of intracellular glutathione (GSH) and the total antioxidant capacity (TAC) of plasma in such patients. Accordingly, our data revealed that monocytes and neutrophils of APS patients had significant losses in mitochondrial membrane potential, indicating that a large proportion of white blood cells contained mitochondria that have lost the capacity to function optimally. Mitochondrial perturbations were related to the autoimmune condition, as well as to the inflammatory and prothrombotic status of APS patients, as suggested by strong positive correlations with the titers of aCL antibodies of IgG isotype, as well as by the association found between the increased percentage of cells with depolarized mitochondria and the heightened occurrence of thrombotic events. Moreover, the presence of an increased CMIT in those patients was associated with that mitochondrial alteration. Parallel in vitro studies indicated that the binding of IgG-APS to the monocytes elicited a redox-sensitive signaling pathway that controlled the prothrombotic phenotype.

Similar studies have demonstrated that antibodies from APS patients are able to up-regulate the expression of the Toll like receptors (TLR) 7 and 8 in plasmacytoid dendritic cells and monocytes respectively, as well as their translocation from the endoplasmic reticulum to the endosome, sensitizing the cells to TLR7 and TLR8 ligands [41]. These effects depend on the uptake of antiphospholipid autoantibodies into the endosome, the activation of NADPH oxidase, and the generation of superoxide.

1.4. Conformations and post-translational redox modifications of β2-glycoprotein I

Oxidative stress plays a direct role in the structure and function of β2-glycoprotein I in patients with APS (reviewed in Giannakopoulos B. and Krilis 2013 [42]). Purified β2GPI is composed of four domains (I through IV) that contain two disulfide bridges each and a fifth domain (domain V) that contains an extra disulfide bridge linking cysteine (Cys) 288 with Cys326. In healthy persons, the free thiol form of β2GPI predominates in the plasma, characterized by a broken disulfide bridge at Cys32 and Cys60 in domain I and another at Cys288 and Cys326 in domain V. The disulfide bridges at these locations are broken by the oxidoreductase thioredoxin-1 and protein disulfide isomerase (PDI). Under conditions of oxidative stress, disulfide bonds form at these sites. The relative proportion of plasma β2GPI in the oxidized versus the free thiol form was significantly greater in patients with APS than in healthy donors [43]. Thus, oxidation unmasks the critical APS B-cell epitope, turning it immunogenic.

Different studies have further demonstrated that β2GPI can potentially exist in a circular form, with domain I interacting with domain V. In this form, the critical B-cell epitope is hidden from the immune system. On binding to an anionic phospholipid surface through domain V, the circular form of β2GPI opens up to a fishhook configuration, exposing the domain I epitope and allowing domain I anti-β2GPI autoantibodies to bind [44].

Those mechanisms could explain why pathogenic antiphospholipid antibodies (aPL) cause the antiphospholipid syndrome (APS) by interacting with domain I (DI) of beta-2-glycoprotein I (β(2)GPI), as well as the ability of the aPL/β(2)GPI complex to exert pathogenic effects on target cells.

1.5. Activation of platelets

It has been shown that β2-glycoprotein I–antibody complexes, but not β2-glycoprotein I alone, can activate different cell types that are involved in the regulation of hemostatic response, resulting in a prothrombotic state. β2-Glycoprotein I can interact with the von Willebrand factor receptor glycoprotein Ibα, and ApoE receptor 2 [45]. This enables anti-β2GPI autoantibodies to cross-link these receptors, leading to the potentiation of platelet activation, the release of thromboxane A2, and an increase in platelet adhesiveness [46]. Platelet factor 4, a cationic protein released by activated platelets, can facilitate the dimerization of β2GPI, enhancing the formation of pathogenic immune complexes on the platelet surface [47].

A very recent study has demonstrated that platelets are the first targets of the β2-glycoprotein I antibody complexes, and products released from activated platelets are responsible for the local activation of endothelial cells [48]. It is also conceivable that these antibody complexes induce the formation of platelet–monocyte complexes. Platelets mediate extravascularization of monocytes with subsequent TF expression of the macrophage. Moreover, monocytes might act as intermediates between platelets and endothelial cells. Therefore, platelets might act as the prime and the in-between target for the β2-glycoprotein I complexes and the leukocytes/endothelial cells.

1.6. Obstetric complications in APS: role for complement activation

Obstetric complications are the second major feature associated with APS. The most common obstetric manifestation of this syndrome is recurrent miscarriage, which is usually defined as three or more consecutive miscarriages before the mid-second trimester, with most losses occurring before the 10th week of gestation. Other obstetric features of APS are one or more fetal deaths occurring at or beyond the 10th week of gestation, severe pre-eclampsia, or placental insufficiency prompting delivery at more than 34 weeks' gestation [49].

Results from studies in mice show a pivotal role for complement activation in fetal loss induced by aPLs [50,51]. Moreover, C4d and C3b fractions are deposited in the placentas of patients with aPLs. Interference with annexin V, a natural anticoagulant, might also favor placental thrombosis and fetal loss [52]. Furthermore, abnormalities in placentation have been described in pregnancy loss related to aPLs [53]. The trophoblasts of the placenta express anionic phospholipids on their cell membrane, enabling them to bind exogenous β2GPI [54]. Moreover, it was also noted that these trophoblasts are capable of synthesizing their own β2GPI [55]. β2GPI directly binds to cultured cytotrophoblast cells and is subsequently recognized by antibodies to β2GPI [56]. The aPL binding reduces the secretion of human chorionic gonadotropin. Moreover, aPLs might trigger an inflammatory response mediated by the Toll-like receptor 4/MyD88 pathway, resulting in trophoblast damage [57].

2. Novel therapeutic options for managing antiphospholipid syndrome

Current therapeutic options for the treatment of the APS remain confined to long-term anticoagulation with vitamin K antagonists, which keep hold of a number of drawbacks, including lack of appropriate answer, increased risk of bleeding, abortions, and recurrences.

As more insight is gained about the pathophysiology of the disease and the involved receptors and intracellular pathways, targeted treatment modalities have been proposed as possible alternatives to the current treatment options. Thus, in the past few years, several potential new therapeutic approaches to APS are emerging, including combination of anti-aggregant therapy, oral antifactor Xa drugs, direct thrombin inhibitors, hydroxychloroquine, and B-cell depletion, among others [49,58].

2.1. New oral anticoagulants

The current mainstay of treatment of thrombotic APS is long-term anticoagulation with oral vitamin K antagonists (VKA) such as warfarin. However, the use of warfarin is problematic, particularly in patients with APS. The new oral anticoagulants include dabigatran etexilate (Pradaxa®, Boehringer Ingelheim Pharma GmbH & Co), a direct thrombin inhibitor, and rivaroxaban (Xarelto®, Bayer Pharma), apixaban

(Eliquis, Bristol-Meyers Squibb Pharmaceuticals) and edoxaban (Lixiana®, Daiichi Sankyo Company), which are direct anti-Xa inhibitors. Unlike warfarin, these agents do not interact with dietary constituents and alcohol, have few reported drug interactions, and monitoring of their anticoagulant intensity is not routinely required due to their predictable anticoagulant effects.

The efficacy of oral direct inhibitor of coagulation (ODI) for venous thromboembolism (VTE) has been demonstrated in large Phase III clinical trials [59–61]. Rivaroxaban, dabigatran, and apixaban have been licensed by the European Medicines Agency (EMA) and approved by the United States Food and Drug Administration (FDA) for several indications. There are no completed studies of ODI in aPL-positive patients. The rivaroxaban in antiphospholipid syndrome (RAPS) trial is the only open label prospective non-inferiority randomized controlled trial (RCT) just developing in patients with thrombotic APS, with or without systemic lupus erythematosus (SLE), who have had either a single episode of VTE while not on anticoagulation or recurrent episode(s) which occurred while off anticoagulation or on subtherapeutic anticoagulant therapy. [62]. The primary aim of the RAPS trial is to demonstrate that the intensity of anticoagulation achieved with rivaroxaban is not inferior to that of warfarin in patients with thrombotic APS.

2.2. Hydroxychloroquine

Hydroxychloroquine (HCQ) has multiple hematologic mechanisms that may contribute to its benefit as an antithrombotic agent, including a reduction in red blood cell slugging, blood viscosity and platelet aggregation. In addition, some of its benefits are not via hematologic pathways, but rather immunologic pathways. These benefits include inhibition of TLRs, reduction in inflammatory cytokines, effects on T cells and neutrophils and reduction in immune complexes [63].

In the setting of APS, HCQ reduces the extent and the time of thrombus persistence in aPL-injected mice [64], reverses thrombogenic properties of aPL in mice, and reverses aPL-mediated platelet activation. HCQ also reduces the attachment of aPL–β2GPI complexes to phospholipid bilayers and cells [66], reverses the binding of aPL to human placental syncytiotrophoblasts, restores annexin A5 expression [65,67], and inhibits Toll-like receptors [68].

Recently, Albert et al. [69] showed that HCQ reversed the aPL-inhibition of trophoblast IL-6 secretion and partially limited aPL-inhibition of cell migration, suggesting that some form of combination therapy that includes HCQ may be beneficial to pregnant APS patients.

The only prospective non-randomized trial comparing oral anticoagulation plus HCQ (400 mg daily) versus oral anticoagulation alone in primary APS patients was recently published. Patients had history of one or two episodes of venous thrombosis and were on anticoagulation with fluindione. No patients received platelet aggregation inhibitors. There were six (30%) venous events in the monotherapy group (n: 20) despite therapeutic range INR and none in the HCQ group (n: 20) during the six month and 36 month follow-up, respectively [70]. Given the small number of patients that were included, the short follow-up, and the methodological limitations of the study, it is difficult to derive meaningful conclusions. Another multicenter, international, prospective, randomized controlled trial of HCQ for primary thrombosis prevention in persistently aPL-positive but thrombosis-free patients without other systemic autoimmune diseases ("HCQ trial") is currently taking place under the auspices of the APS ACTION (www.apsaction.org) (clinicaltrials.gov #:NCT01784523, [71]). The primary objective is to determine the efficacy of HCQ for primary thrombosis prevention over the five year study period. Patients are randomized to receive HCQ or no treatment in addition to their standard regimen.

2.2.1. Statins

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors generically referred to as statins, have emerged as the leading therapeutic regimen for treating hypercholesterolemia and reducing cardiovascular morbidity and mortality. In addition, statins have comprehensive immune-modulating properties that affect many aspects of the inflammatory response. The contribution of TF and proinflammatory mediators to a prothrombotic state in the APS, as well as the proven interference of statins with aPL-mediated thrombosis have provided a renewed focus on antithrombotic therapies in current use.

Several publications have reported the pleiotropic effects of different statins on cultured ECs, platelets, monocytes/macrophages, and in vivo models and human studies of several cardiovascular diseases [72–74]. First, Meroni et al. [75] showed that statins interfere with aPL-induced EC activation via inhibition of the expression of adhesion molecules and IL-6, which is mediated by NFκB. Then, Ferrara et al. [76,77] demonstrated in vivo that fluvastatin inhibited the thrombogenic and inflammatory properties of aPLs and inhibited TF upregulation in aPL-treated ECs. Martínez et al. [78] demonstrated that rosuvastatin decreased expression of VCAM-1 by human umbilical venous endothelial cells exposed to APS serum in an in vitro model. More recently, our group delineated the global effects of fluvastatin on the prothrombotic tendency of monocytes from APS patients [79]. Forty-two APS patients with thrombosis received fluvastatin, 20 mg/d, for 1 month. Blood samples were obtained before the start of treatment, at the end of treatment, and 2 months after the end of treatment. After 1 month of treatment, monocytes showed significant inhibition of TF, PAR-1 and PAR-2, VEGF, and FLT1 expression that was related to the inhibition of p38 MAPK and NFκB/Rel DNA-binding activity. Proteomic analysis further showed proteins involved in thrombotic development (annexin II, RhoA, and protein disulfide isomerase) with altered expression after fluvastatin administration. In vitro studies indicated that the inhibition of HMG-CoA by fluvastatin might inhibit protein prenylation and MAPK activation. Our data agree with those from the study by Redecha et al. [80], which by using a murine model demonstrated the beneficial effects of statins in the setting of APS, showing that statins prevented neutrophil activation by downregulating TF and PAR-2 and protected mouse fetuses from aPL-IgG-induced injury. Moreover, that research group later demonstrated (by using a murine model of recurrent spontaneous miscarriages that shares features with human recurrent miscarriage and fetal growth restriction) that by inhibiting TF with pravastatin, release of antiangiogenic factor sFlt-1 is inhibited, trophoblast proliferation and placental flow are restored, placental oxidative damage is prevented, and pregnancies are rescued [81]. A recent study by Jajoria et al. [82] showed a significant decrease in the titers of VEGF in the plasma of APS patients after 30 days of treatment with fluvastatin. Moreover, that study further addressed the beneficial effects of fluvastatin in other prothrombotic/proinflammatory markers induced by aPLs in APS patients, including TF and TNFα. From these studies, it seems clear that the inhibition of HMG-CoA reductase by fluvastatin, which is a rate-limiting enzyme of the mevalonate pathway, might reduce the expression and activity of specific subfamilies of small GTPases, therefore inhibiting protein prenylation and MAPK activation. This inhibition is likely to have profound effects on key cellular processes, including the suppression of TF and PAR expression, and anti-inflammatory activities on macrophages through the inhibition of proinflammatory cytokines such as VEGF/FLT1. Furthermore, these studies provide significant evidence that fluvastatin has profound and multiple effects in monocyte activity, which might lead to the prevention of thrombosis in APS patients.

Wide experimental evidence found in APS models and the recent randomized clinical trial demonstrating a protective effect for rosuvastatin against first major cardiovascular event in the general population without hyperlipidemia but with elevated high-sensitivity C-reactive protein levels [83] justify clinical studies of statins in aPL-positive patients. Nevertheless, many studies have allowed the qualification of statins as category X by the US Food and Drug Administration and are therefore contraindicated in pregnancy.

2.2.2. Coenzyme Q10

Coenzyme Q10 (CoQ10; ubiquinone) is a vital component of the mitochondrial respiratory chain, with a crucial role in ATP production as the coenzyme for the mitochondrial complexes I, II, and III [84]. CoQ10 provides membrane-stabilizing properties and also acts as an antioxidant with cell-protective effects, including inhibition of LDL oxidation and thus the progression of atherosclerosis. Furthermore, CoQ10 decreases the production of proinflammatory cytokines, as well as blood viscosity, demonstrated to be helpful in patients with heart failure and coronary artery disease.

A recent study by our group [29] demonstrated that autoantibodies from patients with APS can disrupt the mitochondrial function of monocytes and neutrophils, leading to the generation of various intracellular ROS and the subsequent expression of TF and other proinflammatory cytokines. The inhibition of intracellular ROS in monocytes with the use of CoQ10 prevented the upregulation of TF and VEGF/Flt-1 induced by IgG-aPL.

Recent studies suggest that changes in mitochondrial morphology and function may affect a variety of aspects of cardiovascular biology [30], and inhibiting mitochondrial fission has been reported to be cardioprotective [85]. Accordingly, our in vitro study demonstrated 2 beneficial effects of CoQ10, namely, the prevention of mitochondrial dysfunction and oxidative stress and the suppression of the expression of prothrombotic markers relevant to the pathophysiology of APS.

A clinical trial is currently taking place in our hospital under the auspices of Kaneka Corporation to evaluate the beneficial effects of CoQ10 supplementation in the treatment of APS patients.

2.2.3. B-cell depletion

B cells play an important role in APS [86] and are key players in the development, reactivation, and persistence of autoimmune diseases beyond the production of autoantibodies. B-cells orchestrate the immune response by producing antibodies, germinal centers, and cytokines, as well as by their roles in antigen recognition and presentation (independent or dependent of T-cells). B cells are involved in a number aPL related clinical events [86,87] including blocking B-cell activating-factor (BAFF), thus preventing disease onset and prolonging survival in APS murine models [88].

Rituximab is an anti-CD20 chimeric monoclonal antibody that is effective against B-cell non-Hodgkin's lymphomas and chronic lymphocytic leukemias. Several case reports and review articles [89–91] have described rituximab use in APS patients with severe thrombocytopenia [92,93], hemolytic anemia, skin ulcers or necrosis [94], aPL nephropathy [95], and catastrophic APS [96] with variable responses. Rituximab in antiphospholipid syndrome (RITAPS) trial was a pilot open-label Phase II study, the primary objective of which was to evaluate the safety of rituximab in adult APS with no other systemic autoimmune diseases patients (up to 12 months). The RITAPS trial suggested that rituximab in APS patients is safe, and that even without inducing substantial change in aPL profiles, rituximab may be effective in controlling some non-criteria manifestations of aPL [97]. RTX has also been shown to be an effective therapeutic option for life-threatening catastrophic APS in a small number of patients [96]. Interestingly, its use has also been associated with a downregulation of aPL titers [92] and with a reduced rate of recurrent thrombosis in APS patients followed for 10–36 months post-RTX [98].

2.2.4. Complement inhibition

Complement classical pathway, which is initiated by C1q protein is significantly activated in the serum of patients with APS. Activation of the pathway proceeds as cascade reaction producing anaphylatoxins, the fragments of the complement proteins that amplify the activation of monocytes, platelets or endothelial cells. Activation of these cells and molecules induces expression of TF or adhesion molecules and platelet aggregation [99]. Various in vitro and animal models of APS, have demonstrated that: i) interaction of C5a with its receptor C5aR is necessary for aPL-induced placental insufficiency, inflammation, and thrombosis [100]; ii) complement 5a-induced recruitment and activation of neutrophils lead to trophoblast injury and angiogenic factor imbalance in aPL-induced fetal injury [101]; iii) anti-C5 antibody, C5aR antagonist peptides, and complement deficiency experiments prevent pregnancy loss [100]. In addition, inhibition of C5 activation by anti-C5 monoclonal antibodies has been proposed to reduce aPL-mediated prothrombotic status [102]. That heparin has anti-complement effects, in addition to acting as an anticoagulant, may explain some of its efficacy in APS [51].

Clinical studies in APS patients are limited to a small number of case reports. Thus, a case report described improvement of post kidney transplant thrombotic microangiopathy in an APS patient treated with eculizumab (a humanized monoclocal IgG2/4kappa antibody that binds the complement protein C5, preventing cleavage into C5a) [103]. In another catastrophic APS patient resistant to anticoagulation, immunosuppression, plasmapheresis, and rituximab, eculizumab successfully blocked complement activity, aborted progressive thrombosis, and reversed thrombocytopenia [104]. Another case report [105] described catastrophic APS patients who did not respond to eculizumab.

The Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Antibody Syndrome and Systemic Lupus Erythematosus (PROMISSE) [106] is currently evaluating biomarkers that predict pregnancy morbidity and will potentially stimulate interventional trials of complement inhibition in patients at risk of aPL antibody-associated clinical manifestations [107].

2.2.5. Specific GPIIb/IIIa antagonists and other anti-platelet drugs

Antiphospholipid antibodies activate platelets and enhance platelet membrane GPIIb/IIIa expression, a receptor involved in platelet aggregation. In an elegant revision, Erkan and Lockshin described the most recent studies related to inhibition of platelet aggregation [108], which included: 1) the simultaneous administration of anti-GPIIb/IIIa monoclonal antibodies and aPL in mouse models of APS, which resulted in smaller thrombus formation compared with aPL administration only [109]; 2) the prevention of aPL-mediated thrombosis in GPIIb/IIIa deficient mice [110]; and 3) the use of abciximab (a GPIIb/IIIa receptor inhibitor) in the treatment of acute thrombotic syndromes, such as myocardial infarctions and strokes [111]. No data on the use of GPIIa/IIIb receptor inhibitors in APS patients exist, apart from limited data from an uncontrolled study of hydroxychloroquine, which might inhibit aPL-induced GPIIa/IIIB receptor expression [109].

Antiplatelet drugs other than aspirin have been used only rarely in patients with APS. However, combination treatment with aspirin plus dipyridamole and aspirin plus clopidogrel have shown higher efficacy than has aspirin alone in patients with stroke [112] or atrial fibrillation [113], respectively. Such combination might be considered in selected patients with APS in whom warfarin is not effective or safe.

2.2.6. Tissue factor inhibition

Increased TF expression on immune cells including monocytes and neutrophils, as well as the vascular endothelium, seems to play a key role in the pathogenic mechanisms of aPL-mediated thrombosis. Potential pharmacological agents that decrease TF expression include ACE inhibitors, pentoxifyline, an adenosine uptake inhibitor (dilazep), and ss-deoxyribonucleic acid derivatives (e.g. defibrotide, an adenosine receptor agonist) [114]. In the context of cardiovascular disease, several potential mechanisms of TF upregulation have been targeted, including TF synthesis inhibition, TF blockade using anti-TF antibodies or recombinant TF pathway inhibitors [115].

In addition, other well-known anti-thrombotic, anti-oxidant, anti-inflammatory and/or immune-modulators agents have been demonstrated to directly inhibit TF expression including statins, anti-C5 monoclonal antibodies, or coenzyme Q10. Those agents are also discussed in different sections of this review.

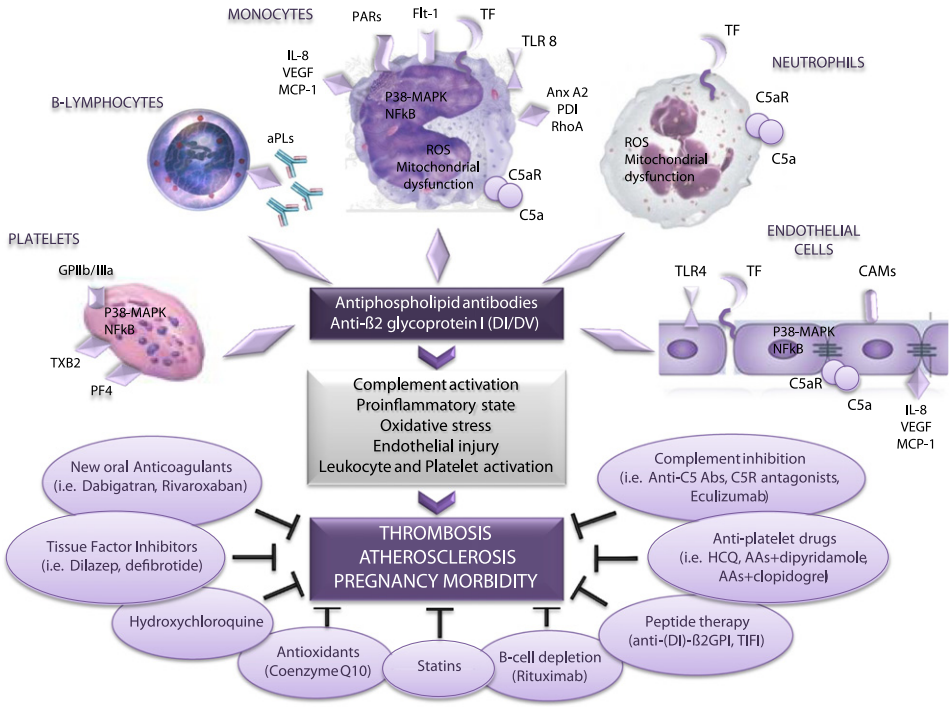

Fig. 1. Mechanisms involved in the aPL-mediated pathogenesis of antiphospholipid syndrome and new therapeutic approaches. Antiphospholipid antibodies (including anti-β2GPI–β2GPI complexes) produced by B-lymphocytes, activate platelets, leukocytes, and endothelial cells: i) monocyte interaction with aPLs promotes the increase in the production of ROS and mitochondrial dysfunction. This process, in parallel with the activation of cell signaling through p38MAPK/NFkB activation, leads to the increased expression of a number of cell surface receptors (i.e. TLRs 7 and 8, PARs 1 and 2, Flt-1, and tissue factor), to the altered expression of annexin A2, protein disulfide isomerase and RhoA proteins, among others, and to the increased secretion of proinflammatory cytokines. Ii) Neutrophil activation leads to complement activation, ROS production, mitochondrial dysfunction and increased TF expression; iii) platelet activation leads to the release of platelet factor 4 and thromboxane-B2, which potentiates the increased expression of GPIIb/IIIa, a major fibrinogen receptor. iv) Endothelial cells activation leads to the increased expression of TLR4 and TF on the cell surface, as well as to the release of proinflammatory cytokines, and increased leukocyte cell adhesion through cell adhesion molecules such as VCAM-1, E-selectin and ICAM-1. The net effect, through the interaction among the activated cells, is the induction of thrombosis, atherosclerosis and pregnancy morbidity, on the background of complement activation and endothelial injury. All that processes might be attenuated, eliminated or reversed trough the combined use of a number of new therapeutic approaches, some of which are still under study in animal models or clinical trials.

2.2.7. Peptide therapy

As above mentioned, anti-DI anti-β2-glycoprotein-1 antibodies are most closely related to pathogenicity in APS, but binding of DV to its receptor is required as well. Proposed peptide therapies mainly target the DI–aPL interaction or the DV–phospholipid interaction.

Preliminary results showed that a recombinant DI molecule, and a recombinant mutant DI with enhanced aPL binding properties, may be used as an inhibitor of aPL binding, and thus inhibit aPL-induced pathogenesis [116].

Other studies have focused on targeting the DV-phospholipid interaction. Among them, Pierangeli and coworkers [117] investigated the role of TIFI, a 20 amino acid synthetic peptide that shares similarity with the domain V of β2GPI in APS. They observed that TIFI reduced aPL-mediated thrombosis in mice by competing with β2GPI and preventing its binding to target cells. De la Torre et al. showed that TIFI inhibits binding of aPL to human trophoblast cells in vitro and also reduces fetal loss in mice induced by injection of aPL [118]. There are no peptide therapies currently available or in trials for aPL-positive patients.

3. Conclusions

Antiphospholipid syndrome (APS) is a systemic autoimmune vascular disease characterized by recurrent thrombotic episodes and/or obstetric complications. In recent years, significant advancement has been made in elucidating the pathophysiology of the disease including antiphospholipid antibody (aPL)-induced activation of the platelets, endothelial cells, monocytes, complement and coagulation cascade (Fig. 1). These achievements in our understanding of the disease have opened the door to the possibility of new more targeted therapeutic options that might be safer and more efficacious than the standard treatment modalities.

New anticoagulant agents, hydroxychloroquine, statins, rituximab, complement inhibitors, coenzyme Q10 and biologic agents with selected aPL-related targets will potentially be part of APS management in the future. Probably the best therapeutic strategy would imply a combination of anticoagulant, immunomodulatory, and anti-inflammatory drugs, which should further be administered taking into account the clinical and molecular profile specific of each APS patient. Large multicenter, randomized clinical trials are needed to confirm and expand evidence regarding the efficacy of various therapeutic interventions.

Abbreviations

aPL	antiphospholipid antibodies
C5a	complement protein 5 activated
C5aR	C5a receptor
p38 MAPK	p38 mitogen-activated protein kinase
NF-kB	nuclear factor kappa B
ROS	reactive oxygen species
TLRs	Toll-like receptors
TF	tissue factor
PARs	protease activated receptors
PDI	protein disulfide isomerase
AnxA2	annexin A 2
RhoA	rho A proteins
TXB2	thromboxane B2
PF4	platelet factor 4
GPIIb/IIIa	glycoprotein IIb IIIa
CAM	cell adhesion molecule

IL-8	interleukin 8
VEGF	vascular endothelial growth factor
Flt1	FMS-like tyrosine kinase 1 (VEGF-receptor 1)
MCP-1	monocyte chemoattractant protein 1

Acknowledgments

This work was supported by grants from the 'Junta de Andalucía' (CTS-7940), the Ministry of Health, co-financed with FEDER funds (PI12/01511), and the Spanish foundation of Rheumatology. CL-P was supported by a contract from the Spanish Junta de Andalucía.

References

[1] M.J. Cuadrado, C. Lopez-Pedrera, M.A. Khamashta, et al., Thrombosis in primary antiphospholipid syndrome: a pivotal role for monocyte tissue factor expression, Arthritis Rheum. 40 (1997) 834–841.
[2] P.M. Dobado-Berrios, Ch. Lopez-Pedrera, F. Velasco, et al., Increased levels of tissue factor mRNA in mononuclear blood cells of patients with primary antiphospholipid syndrome, Thromb. Haemost. 82 (1999) 1578–1582.
[3] P.M. Dobado-Berrios, Ch. Lopez-Pedrera, F. Velasco, M.J. Cuadrado, The role of TF in the antiphospholipid syndrome, Arthritis Rheum. 44 (2001) 2467–2476.
[4] Ch. López-Pedrera, P. Buendía, M.J. Cuadrado, et al., Antiphospho-lipid antibodies from antiphospholipid syndrome patients induce monocyte expression through the simultaneous activation of both NFkB/Rel proteins via p38 MAPK pathway, and the MEK1/ERK pathway, Arthritis Rheum. 54 (2006) 301–311.
[5] M. Vega-Ostertag, E.N. Harris, S.S. Pierangeli, Intracellular events in platelet activation induced by antiphospholipid antibodies in the presence of low doses of thrombin, Arthritis Rheum. 50 (2004) 2911–2919.
[6] M. Bohgaki, T. Atsumi, Y. Yamashita, et al., The p38 mitogen-activated protein kinase (MAPK) pathway mediates induction of the tissue factor gene in monocytes stimulated with human monoclonal anti-beta2 glycoprotein I antibodies, Int. Immunol. 16 (2004) 1633–1641.
[7] G. Montiel-Manzano, Z. Romay-Penabad, E. Papalardo de Martinez, et al., In vivo effects of an inhibitor of nuclear factor-kappa B on thrombogenic properties of antiphospholipid antibodies, Ann. N. Y. Acad. Sci. 1108 (2007) 540–553.
[8] S. Dunoyer-Geindre, P. de Moerloose, B. Galve-de Rochemonteix, et al., NFkappa B is an essential intermediate in the activation of endothelial cells by anti-beta(2)-glycoprotein 1 antibodies, Thromb. Haemost. 88 (2002) 851–857.
[9] M. Vega-Ostertag, K. Casper, R. Swerlick, et al., Involvement of p38 MAPK in the upregulation of tissue factor on endothelial cells by antiphospholipid antibodies, Arthritis Rheum. 52 (2005) 1545–1554.
[10] H.F. Dvorak, Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy, J. Clin. Oncol. 20 (2002) 4368–4380.
[11] F.M.K. Williams, K. Parmar, G.R.V. Hughes, B.J. Hunt, Systemic endothelial cell markers in primary antiphospholipid syndrome, Thromb. Haemost. 84 (2000) 742–746.
[12] M.J. Cuadrado, P. Buendía, F. Velasco, et al., Vascular endothelial growth factor expression in monocytes from patients with primary antiphospholipid syndrome, J. Thromb. Haemost. 4 (2006) 2461–2469.
[13] C. López-Pedrera, M.A. Aguirre, P. Buendía, et al., Differential expression of protease activated receptors in monocytes from patients with primary antiphospholipid syndrome, Arthritis Rheum. 62 (2010) 869–877.
[14] C. López-Pedrera, M.J. Cuadrado, V. Hernández, et al., Proteomic analysis in monocytes of antiphospholipid syndrome patients: deregulation of proteins related to the development of thrombosis, Arthritis Rheum. 58 (2008) 2835–2844.
[15] N.J. Goulding, J. Dixey, E.F. Morand, R.A. Dodds, L.S. Wilkinson, A.A. Pitsillides, et al., Differential distribution of annexins-I, -II, -IV, and -VI in synovium, Ann. Rheum. Dis. 54 (1995) 841–845.
[16] D.J. Falcone, W. Borth, K.M. Faisal Khan, K.A. Hajjar, Plasminogen mediated matrix invasion and degradation by macrophages is dependent on surface expression of annexin II, Blood 97 (2001) 777–784.
[17] J. Ahamed, H.H. Versteeg, M. Kerver, V.M. Chen, B.M. Mueller, P.J. Hogg, et al., Disulfide isomerization switches TF from coagulation to cell signaling, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 13932–13937.
[18] T. Higuchi, Y. Watanabe, I. Waga, Protein disulfide isomerase suppresses the transcriptional activity of NFkB, Biochem. Biophys. Res. Commun. 318 (2004) 46–52.
[19] J. George, D. Harats, B. Gilburd, et al., Immunolocalization of beta2-glycoprotein I (apolipoprotein H) to human atherosclerotic plaques: potential implications for lesion progression, Circulation 99 (1999) 2227–2230.
[20] Y. Shoenfeld, Y. Sherer, J. George, D. Harats, Autoantibodies associated with atherosclerosis, Ann. Med. 32 (2000) 37–40.
[21] L.J. Jara, G. Medina, O. Vera-Lastra, Systemic antiphospholipid syndrome and atherosclerosis, Clin. Rev. Allergy Immunol. 32 (2007) 172–177.
[22] P. Soltész, K. Veres, G. Lakos, E. Kiss, L. Muszbek, G. Szegedi, Evaluation of clinical and laboratory features of antiphospholipid syndrome: a retrospective study of 637 patients, Lupus 12 (4) (2003) 302–307.
[23] S. Jimenez, M.A. García-Criado, D. Tassies, et al., Preclinical vascular disease in systemic lupus erythematosus and primary antiphospholipid syndrome, Rheumatology 44 (6) (2005) 756–761.
[24] Y. Hasunuma, E. Matsuura, Z. Makita, T. Katahira, S. Nishi, T. Koike, Involvement of beta2-glycoprotein I and anticardiolipin antibodies in oxidatively modified low-density lipoprotein uptake by macrophages, Clin. Exp. Immunol. 107 (3) (1997) 569–573.
[25] O. Vaarala, Antiphospholipid antibodies and atherosclerosis, Lupus 5 (5) (1996) 442–447.
[26] K. Veres, G. Lakos, A. Kerenyi, et al., Antiphospholipid antibodies in acute coronary syndrome, Lupus 13 (6) (2004) 423–427.
[27] M. Cugno, M.O. Borghi, L.M. Lonati, et al., Patients with antiphospholipid syndrome display endothelial perturbation, J. Autoimmun. 34 (2) (2010) 105–110.
[28] P.R.J. Ames, I. Antionolfi, G. Scenna, G. Gaeta, M. Margagliones, A. Margarita, Atherosclerosis in thrombotic primary antiphospholipid syndrome, J. Thromb. Haemost. 7 (4) (2009) 537–542.
[29] C. Perez-Sanchez, P. Ruiz-Limon, M.A. Aguirre, M.L. Bertolaccini, M.A. Khamashta, A. Rodriguez-Ariza, et al., Mitochondrial dysfunction in antiphospholipid syndrome: implications in the pathogenesis of the disease and effects of coenzyme Q(10) treatment, Blood 119 (24) (Jun 14 2012) 5859–5870.
[30] P.R. Ames, A. Margarita, J. Delgado Alves, C. Tommasino, L. Iannaccone, V. Brancaccio, Anticardiolipin antibody titre and plasma homocysteine level independently predict intima media thickness of carotid arteries in subjects with idiopathic antiphospholipid antibodies, Lupus 11 (4) (2002) 208–214.
[31] P.R. Ames, A. Margarita, K.B. Sokoll, M. Weston, V. Brancaccio, Premature atherosclerosis in primary antiphospholipid syndrome: preliminary data, Ann. Rheum. Dis. 64 (2) (2005) 315–317.
[32] A. Margarita, J. Batuca, G. Scenna, et al., Subclinical atherosclerosis in primary antiphospholipid syndrome, Ann. N. Y. Acad. Sci. 1108 (2007) 475–480.
[33] D. Shah, A. Wanchu, A. Bhatnagar, Interaction between oxidative stress and chemokines: possible pathogenic role in systemic lupus erythematosus and rheumatoid arthritis, Immunobiology 216 (9) (2011) 1010–1017.
[34] A.D. Luster, Chemokines–chemotactic cytokines that mediate inflammation, N. Engl. J. Med. 338 (7) (1998) 436–445.
[35] K. Marutsuka, K. Hatakeyama, A. Yamashita, Y. Asada, Role of thrombogenic factors in the development of atherosclerosis, J. Atheroscler. Thromb. 12 (1) (2005) 1–8.
[36] A. Perl, Pathogenic mechanisms in systemic lupus erythematosus, Autoimmunity 43 (1) (2010) 1–6.
[37] J.D. Alves, B. Grima, Oxidative stress in systemic lupus erythematosus and antiphospholipid syndrome: a gateway to atherosclerosis, Curr. Rheumatol. Rep. 5 (5) (2003) 383–390.
[38] J.D. Morrow, L.J. Roberts, The isoprostanes. Current knowledge and directions for future research, Biochem. Pharmacol. 51 (1) (1996) 1–9.
[39] L. Iuliano, D. Practico, D. Ferro, et al., Enhanced lipid peroxidation in patients positive for antiphospholipid antibodies, Blood 90 (10) (1997) 3931–3935.
[40] M. Charakida, C. Besler, J.R. Batuca, et al., Vascular abnormalities, paraoxonase activity, and dysfunctional HDL in primary antiphospholipid syndrome, JAMA 302 (11) (2009) 1210–1217.
[41] N. Prinz, N. Clemens, D. Strand, et al., Antiphospholipid antibodies induce translocation of TLR7 and TLR8 to the endosome in human monocytes and plasmacytoid dendritic cells, Blood 118 (2011) 2322–2332.
[42] CONFORMATIONS OF B2GPI. B. Giannakopoulos, A. Krilis, The pathogenesis of the Antiphospholipid syndrome, N. Engl. J. Med. 368 (2013) 1033–1044.
[43] Y. Ioannou, J.Y. Zhang, M. Qi, L. Gao, J.C. Qi, D.M. Yu, et al., Novel assays of thrombogenic pathogenicity in the antiphospholipid syndrome based on the detection of molecular oxidative modification of the major autoantigen β2-glycoprotein I, Arthritis Rheum. 63 (2011) 2774–2782.
[44] C. Agar, G.M. van Os, M. Morgelin, et al., Beta2-glycoprotein I can exist in 2 conformations: implications for our understanding of the antiphospholipid syndrome, Blood 116 (2010) 1336–1343.
[45] R.T. Urbanus, M.T. Pennings, R.H. Derksen, P.G. de Groot, Platelet activation by dimeric beta2-glycoprotein I requires signalling via both glycoprotein Ibalpha and apolipoprotein E receptor 2′, J. Thromb. Haemost. 6 (2008) 1405–1412.
[46] T. Shi, B. Giannakopoulos, X. Yan, 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. 54 (2006) 2558–2567.
[47] M.P. Sikara, J.G. Routsias, M. Samiotaki, G. Panayotou, H.M. Moutsopoulos, P.G. Vlachoyiannopoulos, {beta}2 Glycoprotein I ({beta}2GPI) binds platelet factor 4 (PF4): implications for the pathogenesis of antiphospholipid syndrome, Blood 115 (3) (Jan 21 2010) 713–723.
[48] V. Proulle, R.A. Furie, G. Merrill-Skoloff, B.C. Furie, B. Furie, Platelets are required for enhanced activation of the endothelium and fibrinogen in a mouse thrombosis model of APS, Blood 124 (4) (Jul 24 2014) 611–622.
[49] G. Ruiz-Irastorza, M. Crowther, W. Branch, M.A. Khamashta, Antiphospholipid syndrome, Lancet 376 (2010) 1498–1509.
[50] S.S. Pierangeli, G. Girardi, M. Vega-Ostertag, et al., Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia, Arthritis Rheum. 52 (2005) 2120–2124.
[51] G. Girardi, P. Redecha, J.E. Salmon, Heparin prevents antiphospho-lipid antibody-induced fetal loss by inhibiting complement activation, Nat. Med. 10 (2004) 1222–1226.
[52] S.S. Pierangeli, P.P. Chen, E. Raschi, et al., Antiphospholipid antibodies and the antiphospholipid syndrome: pathogenic mechanisms, Semin. Thromb. Hemost. 34 (2008) 236–250.
[53] S. Stone, M.A. Khamashta, L. Poston, Placentation, antiphospholipid syndrome and pregnancy outcome, Lupus 10 (2001) 67–74.
[54] N. Di Simone, M.P. Luigi, D. Marco, et al., Pregnancies complicated with antiphospholipid syndrome: the pathogenic mechanism of antiphospholipid antibodies: a review of the literature, Ann. N. Y. Acad. Sci. 1108 (2007) 505–514.

[55] L.W. Chamley, J.L. Allen, P.M. Johnson, Synthesis of beta2 glycoprotein 1 by the human placenta, Placenta 18 (1997) 403–410.
[56] N. Di Simone, E. Raschi, C. Testoni, et al., Pathogenic role of anti-beta 2-glycoprotein I antibodies in antiphospholipid associated fetal loss: characterisation of beta 2-glycoprotein I binding to trophoblast cells and functional effects of anti-beta 2-glycoprotein I antibodies in vitro, Ann. Rheum. Dis. 64 (2005) 462–467.
[57] M.J. Mulla, J.J. Brosens, L.W. Chamley, et al., Antiphospholipid antibodies induce a pro-inflammatory response in first trimester trophoblast via the TLR4/MyD88 pathway, Am. J. Reprod. Immunol. 62 (2009) 96–111.
[58] D. Erkan, C.L. Aguiar, A. Andrade, H. Cohen, M.J. Cuadrado, A. Danowski, et al., 14th International Congress on Antiphospholipid antibodies task force report on Antiphospholipid syndrome treatment trends, Autoimmun. Rev. 13 (2014) 685–696.
[59] G. Agnelli, H.R. Buller, A. Cohen, M. Curto, A.S. Gallus, M. Johnson, et al., Oral apixaban for the treatment of acute venous thromboembolism, N. Engl. J. Med. 368 (2013) 799–808.
[60] EINSTEIN Investigators, R. Bauersachs, S.D. Berkowitz, B. Brenner, H.R. Buller, H. Decousus, et al., Oral rivaroxaban for symptomatic venous thromboembolism (and supplementary appendix), N. Engl. J. Med. 363 (2010) 2499–2510.
[61] Investigators Hokusai-VTE, H.R. Büller, H. Décousus, M.A. Grosso, M. Mercuri, S. Middeldorp, et al., Edoxaban versus warfarin for the treatment of symptomatic venous thromboembolism, N. Engl. J. Med. 369 (2013) 1406–1415.
[62] Summary of product characteristics (SPC), EU. Xarelto 10 mg film-coated tablets. Bayer HealthCare AG. Date of first authorization: 09/30/2008. Date of renewal of authorization: 05/22/2013. Date of revision of text: 06/2013. Available from: http://www.medicines.org.uk/emc/medicine/21265/SPC/Xarelto+10+mg+filmcoated+tablets.
[63] M. Petri, Use of hydroxychloroquine to prevent thrombosis in systemic lupus erythematosus and in antiphospholipid antibody-positive patients, Curr. Rheumatol. Rep. 13 (2011) 77–80.
[64] M.H. Edwards, S. Pierangeli, X. Liu, J.H. Barker, G. Anderson, E.N. Harris, Hydroxychloroquine reverses thrombogenic properties of antiphospholipid antibodies in mice, Circulation 96 (1997) 4380–4384.
[65] J.H. Rand, X.X. Wu, A.S. Quinn, P.P. Chen, J.J. Hathcock, D.J. Taatjes, Hydroxychloroquine directly reduces the binding of antiphospholipid antibody–beta2-glycoprotein I complexes to phospholipid bilayers, Blood 112 (2008) 1687–1695.
[66] J.H. Rand, X.X. Wu, A.S. Quinn, A.W. Ashton, P.P. Chen, J.J. Hathcock, 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 115 (2010) 2292–2299.
[67] X.X. Wu, S. Guller, J.H. Rand, Hydroxychloroquine reduces binding of antiphospholipid antibodies to syncytiotrophoblasts and restores annexin A5 expression, Am. J. Obstet. Gynecol. 205 (576) (2011) e7–e14.
[68] K. Sacre, L.A. Criswell, J.M. Mc Cune, Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic cells in systemic lupus erythematosus, Arthritis Res. Ther. 14 (2012) R155.
[69] C.R. Albert, W.J. Schlesinger, C.A. Viall, Effect of hydroxychloroquine on antiphospholipid antibody-induced changes in first trimester trophoblast function, Am. J. Reprod. Immunol. 71 (2014) 154–164.
[70] A. Schmidt-Tanguy, J. Voswinkel, D. Henrion, J.F. Subra, L. Loufrani, V. Rohmer, N. Ifrah, C. Belizna, Antithrombotic effects of hydroxychloroquine in primary antiphospholipid syndrome patients, J. Thromb. Haemost. 11 (10) (Oct 2013) 1927–1929.
[71] Hydroxychloroquine for the First Thrombosis Prevention in Antiphospholipid Antibody Positive Patients, IRSCTN NCT01784523, (Feb 3 2013).
[72] A. Undas, E. Brummel-Ziedins, K.G. Mann, Statins and blood coagulation, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 1–8.
[73] A. Undas, M. Celiska-Lowenhoff, M. Kaczor, J. Musial, New nonlipid effects of statins and their clinical relevance in cardiovascular disease, Thromb. Haemost. 91 (2004) 1065–1077.
[74] R. Krysiak, B. Okopien, Z.S. Herman, Effects of HMG-CoA reductase inhibitors on coagulation and fibrinolysis processes, Drugs 63 (2003) 1821–1854.
[75] P.L. Meroni, E. Raschi, C. Testoni, et al., Statins prevent endothelial cell activation induced by antiphospholipid (anti-beta2-glycoprotein I) antibodies: effect on the proadhesive and proinflammatory phenotype, Arthritis Rheum. 44 (2001) 2870–2878.
[76] D.E. Ferrara, X. Liu, R.G. Espinola, et al., Inhibition of the thrombogenic and inflammatory properties of antiphospholipid antibodies by fluvastatin in an in vivo animal model, Arthritis Rheum. 48 (2003) 3272–3279.
[77] D.E. Ferrara, R. Swerlick, K. Casper, et al., Fluvastatin inhibits up-regulation of tissue factor expression by antiphospholipid anti-bodies on endothelial cells, J. Thromb. Haemost. 2 (2004) 1558–1563.
[78] L.A. Martínez, M.C. Amigo, A. Orozco, et al., Effect of rosuvastatin on VCAM-1 expression by HUVEC exposed to APS serum in an in vitro model, Clin. Exp. Rheumatol. 25 (2007) 18–19.
[79] C. López-Pedrera, P. Ruiz-Limón, M.A. Aguirre, et al., Global effects of fluvastatin on the prothrombotic status of patients with antiphospholipid syndrome, Ann. Rheum. Dis. 70 (2011) 675–682.
[80] P. Redecha, C.W. Franzke, W. Ruf, et al., Neutrophil activation by the tissue factor/factor VIIa/PAR2 axis mediates foetal death in a mouse model of antiphospholipid syndrome, J. Clin. Invest. 118 (2008) 3453–3461.
[81] P. Redecha, N. van Rooijen, D. Torry, G. Girardi, Pravastatin prevents miscarriages in mice: role of tissue factor in placental and foetal injury, Blood 113 (2009) 4101–4109.
[82] P. Jajoria, V. Murthy, E. Papalardo, et al., Statins for the treatment of antiphospholipid syndrome? Ann. N. Y. Acad. Sci. 1173 (2009) 736–745.
[83] P.M. Ridker, E. Danielson, F.A. Fonseca, Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein, N. Engl. J. Med. 20 (2008) 2195–2207.
[84] M. Turunen, J. Olsson, G. Dallner, Metabolism and function of coenzyme Q, Biochim. Biophys. Acta 1660 (1–2) (2004) 171–199.
[85] S.B. Ong, S. Subrayan, S.Y. Lim, D.M. Yellon, S.M. Davidson, D.J. Hausenloy, Inhibiting mitochondrial fission protects the heart against ischemia/ reperfusion injury, Circulation 121 (18) (2010) 2012–2022.
[86] P. Youinou, Y. Reneaudineau, The antiphospholipid syndrome as a model for B cell-induced autoimmune diseases, Thromb. Res. 114 (2004) 363–369.
[87] S. Khattri, G. Zandman-Goddard, E. Peeva, B-cell directed therapies in antiphospholipid antibody syndrome—new directions based on murine and human data, Autoimmun. Rev. 11 (2012) 717–722.
[88] P. Kahn, M. Ramanujman, R. Bethunaickan, W. Huang, H. Tao, M.P. Madaio, et al., Prevention of murine antiphospholipid syndrome by BAFF blockade, Arthritis Rheum. 58 (2008) 2824–2834.
[89] D. Kumar, R.A.S. Roubey, Use of rituximab in the antiphospholipid syndrome, Curr. Rheumatol. Rep. 12 (2010) 40–44.
[90] E. Rubenstein, D.G. Arkfeld, S. Metyas, S. Shinada, S. Ehresmann, H.A. Liebman, Rituximab treatment for resistant antiphospholipid syndrome, J. Rheumatol. 33 (2006) 355–357.
[91] F. Tenedios, D. Erkan, M.D. Lockshin, Rituximab in the Primary Antiphospholipid Syndrome (PAPS), Arthritis Rheum. 52 (2005) 4078.
[92] S. Sciascia, C. Naretto, D. Rossi, M. Bazzan, D. Roccatello, Treatment-induced down-regulation of antiphospholipid antibodies: effect of rituximab alone on clinical and laboratory features of antiphospholipid syndrome, Lupus 20 (2011) 1106–1108.
[93] R. Trappe, A. Loew, P. Thuss-Patience, B. Dorken, H. Riess, Successful treatment of thrombocytopenia in primary antiphospholipid antibody syndrome with the anti-CD20 antibody rituximab—monitoring of antiphospholipid and anti-GP antibodies: a case report, Ann. Hematol. 85 (2006) 134–135.
[94] R. Costa, S. Fazal, R.B. Kaplan, J. Spero, R. Costa, Successful plasma exchange combined with rituximab therapy in aggressive APS-related cutaneous necrosis, Clin. Rheumatol. 32 (Suppl. 1) (2013) S79–S82, http://dx.doi.org/10.1007/s10067-010-1506-3.
[95] G. Tsagalis, E. Psimenou, L. Nakopoulou, A. Laggouranis, Combination treatment with plasmapheresis and rituximab for recurrent focal segmental glomerulosclerosis after renal transplantation, Artif. Organs 35 (2011) 420–425.
[96] H. Berman, I. Rodríguez-Pintó, R. Cervera, N. Morel, N. Costedoat-Chalumeau, D. Erkan, et al., Catastrophic Antiphospholipid Syndrome (CAPS) Registry Project Group (European Forum on Antiphospholipid Antibodies). Rituximab use in the catastrophic antiphospholipid syndrome: Descriptive analysis of the CAPS registry patients receiving rituximab, Autoimmun. Rev. 12 (2013) 1085–1090, http://dx.doi.org/10.1016/j.autrev.2013.05.004.
[97] D. Erkan, J. Vega, G. Ramon, E. Kozora, M.D. Lockshin, Rituximab in antiphospholipid syndrome (RITAPS) — a pilot open-label phase II prospective trial for non-criteria manifestations of antiphospholipid antibodies, Arthritis Rheum. 65 (2013) 464–471.
[98] J. Bakshi, R. Stevens, Rituximab therapy for recurrent thromboembolic disease in antiphospholipid syndrome, Lupus 22 (2013) 865–867.
[99] K. Oku, O. Amengual, T. Atsumi, Pathophysiology of thrombosis and pregnancy morbidity in the antiphospholipid syndrome, Eur. J. Clin. Investig. 42 (10) (Oct 2012) 1126–1135.
[100] G. Girardi, J. Berman, P. Redecha, L. Spruce, J.M. Thurman, D. Kraus, et al., Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome, J. Clin. Invest. 112 (2003) 1644–1654.
[101] P. Redecha, R. Tiley, M. Tencati, J.E. Salmon, D. Kirchhofer, N. Mackman, et al., Tissue factor: a link between C5a and neutrophil activation in antiphospholipid antibody induced fetal injury, Blood 110 (2007) 2423–2431.
[102] S.S. Pierangeli, M. Vega-Ostertag, X. Liu, G. Girardi, Complement activation: a novel pathogenic mechanism in the antiphospholipid syndrome, Ann. N. Y. Acad. Sci. 1051 (2005) 413–420.
[103] B.E. Lonze, A.L. Singer, R.A. Montgomery, Eculizumab and renal transplantation in a patient with CAPS, N. Engl. J. Med. 362 (2010) 1744–1745.
[104] I. Shapira, D. Andrade, S.L. Allen, J.E. Salmon, Brief report: induction of sustained remission in recurrent catastrophic antiphospholipid syndrome via inhibition of terminal complement with eculizumab, Arthritis Rheum. 64 (2012) 2719–2723.
[105] R. Khianey, S. Mushin, D. Erkan, Discordant aPTT and anti-FXa values in a catastrophic antiphospholipid syndrome patient receiving intravenous unfractionated heparin, APLA-LACA2013. (Abstract).
[106] Predictors of Pregnancy Outcome in Systemic Lupus Erythematosus (SLE) and Antiphospholipid Syndrome (APS) (PROMISSE) Accession date 05/01/2014, ClinicalTrials.gov Identifier: NCT00198068, (Sep 12, 2005).
[107] J.E. Salmon, G. Girardi, E. Theodore, Woodward Award: antiphospholipid syndrome revisited: a disorder initiated by inflammation, Trans. Am. Clin. Climatol. Assoc. 118 (2007) 99–114.
[108] D. Erkan, M.D. Lockshin, New approaches for managing antiphospholipid syndrome, Nat. Clin. Pract. Rheumatol. 5 (3) (Mar 2009) 160–170.
[109] S.S. Pierangeli, M. Vega-Ostertag, E.N. Harris, Intracellular signaling triggered by antiphospholipid antibodies in platelets and endothelial cells: a pathway to targeted therapies, Thromb. Res. 114 (2004) 467–476.
[110] S.S. Pierangeli, M.E. Vega-Ostertag, E.B. González, New targeted therapies for treatment of thrombosis in antiphospholipid syndrome, Expert Rev. Mol. Med. 9 (2007) 1–15.
[111] A. Straub, H.P. Wendel, R. Azevedo, G. Ziemer, The GP IIb/IIIa inhibitor abciximab (ReoPro) decreases activation and interaction of platelets and leukocytes during

in vitro cardiopulmonary bypass simulation, Eur. J. Cardiothorac. Surg. 27 (2005) 617–621.
[112] ESPRIT Study Group, P.H. Halkes, J. van Gijn, L.J. Kappelle, P.J. Koudstaal, A. Algra, Aspirin plus dipyridamole versus aspirin alone after cerebral ischaemia of arterial origin (ESPRIT): randomised controlled trial, Lancet 367 (2006) 1665–1673.
[113] ACTIVE Investigators, Effect of clopidogrel added to aspirin in patients with atrial fibrillation, N. Engl. J. Med. 360 (2009) 2066–2078.
[114] H. Zhou, A.S. Wolberg, R.A. Roubey, Characterization of monocyte tissue factor activity induced by IgG antiphospholipid antibodies and inhibition by dilazep, Blood 104 (8) (Oct 15 2004) 2353–2358.
[115] E.W. Holy, F.C. Tanner, Tissue factor in cardiovascular disease pathophysiology and pharmacological intervention, Adv. Pharmacol. 59 (2010) 259–292.
[116] Y. Ioannou, Z. Romay-Penabad, C. Pericleous, et al., In vivo inhibition of antiphospholipid antibody-induced pathogenicity utilizing the antigenic target peptide domain I of beta2-glycoprotein I: proof of concept, J. Thromb. Haemost. 7 (2009) 833–842.
[117] M.V. Ostertag, X. Liu, V. Henderson, S.S. Pierangeli, A peptide that mimics the Vth region of beta-2-glycoprotein I reverses antiphospholipid-mediated thrombosis in mice, Lupus 15 (2006) 358–365.
[118] Y.M. de la Torre, F. Pregnolato, F. D'Amelio, C. Grossi, N. Di Simone, F. Pasqualini, et al., Anti-phospholipid induced murine fetal loss: novel protective effect of a peptide targeting the beta2 glycoprotein I phospholipid-binding site. Implications for human fetal loss, J. Autoimmun. 38 (2012) J209–J215.