The Pathophysiology of The Antiphospholipid Syndrome: A Perspective From The Blood Coagulation System

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Abstract

The antiphospholipid syndrome (APS), a systemic autoimmune disease characterized by a hypercoagulability associated to vascular thrombosis and/or obstetric morbidity, is caused by the presence of antiphospholipid antibodies such as lupus anticoagulant, anti-β-2-glycoprotein 1, and/or anticardiolipin antibodies. In the obstetrical APS, antiphospholipid antibodies induce the production of proinflammatory cytokines and tissue factor by placental tissues and recruited neutrophils. Moreover, antiphospholipid antibodies activate the complement system which, in turn, induces a positive feedback leading to recruitment of neutrophils as well as activation of the placenta. Activation of these cells triggers myometrial contractions and cervical ripening provoking the induction of labor. In thrombotic and obstetrical APS, antiphospholipid antibodies activate endothelial cells, platelets, and neutrophils and they may alter the multimeric pattern and concentration of von Willebrand factor, increase the concentration of thrombospondin 1, reduce the inactivation of factor XI by antithrombin, increase the activation of factor XII, and reduce the activity of tissue plasminogen activator with the subsequent production of plasmin. All these effects result in less permeable clots, denser, thinner, and with more branched fibrin fibers which are more difficult to lysate. As a consequence, thrombosis, the defining clinical criterion of APS, complicates the clinical course of the patient.

1. Antiphospholipid Syndrome

1.1. Definition, Classification, and Clinical Aspects

The antiphospholipid syndrome (APS) is a systemic hematological autoimmune disease characterized by a hypercoagulable state which is associated with vascular thrombosis and/or obstetric morbidity characterized by miscarriage, fetal death, and/or premature birth. The APS is associated to the presence of antiphospholipid antibodies (aPLs) such as lupus anticoagulant (LA) anti-β-2 glycoprotein 1 (α-β2GP1), and anticardiolipin antibodies (aCL).[1–4]

APS is classified as primary when no clinical or laboratory evidence of another disease is found in the patient; secondary APS is defined when another clinical condition like an autoimmune disease, infection, drugs and/or cancer is present.[3,5]

Secondary APS can occur in association with hemolytic anemia,[6,7] immune thrombocytopenic purpura,[8] juvenile arthritis,[3] rheumatoid arthritis,[9] psoriatic arthritis,[3] systemic sclerosis,[10]

Behçet syndrome,[11] Sjögren syndrome,[12] polymyositis,[13] dermatoses,[13] rheumatic polymyalgia,[14] systemic lupus erythematosus,[15] eosinophilic myalgia,[3] vasculitis[8] and autoimmune thyroid disease[16] in which the presence of aCL and/or LA have been described. Furthermore, secondary APS has been associated with

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infectious diseases which trigger the production of aPLs capable of inducing some clinical manifestations such as the catastrophic antiphospholipid syndrome. In 2004, 100 infection-associated APS patients and their infections were analyzed. The infections were located at the skin (18%), urinary tract (10%), upper respiratory tract (9%), sepsis (6%), and gastrointestinal tract (6%). On the other hand, most frequently associated etiological agents were HIV (17%), varicella-zoster virus (15%), and hepatitis C virus (13%), among others. In some cases, more than one infection was associated to the secondary APS.[17] Drugs like chlorpromazine, phenytoin, hydralazine, procainamide,[18] quinidine, ethosuximide, interferon-alpha, amoxicillin, chlorothiazide, oral contraceptives,[19] cysteamine,[20] and propranolol have also been described as inductors of aPLs and the further development of APS. It has been documented that tumor necrosis factor-α (TNF-α) inhibitors may induce the production of aPLs.[3]

Table 1 shows the main clinical manifestations of APS. In APS, thrombosis may complicate any vascular bed.[21] Based on the clinical manifestations, APS is divided into obstetrical and thrombotic APS, albeit some patients may display both clinical signs. A deep review of the pathogenic mechanisms of both types of APS will be addressed later in the text.

1.2. Diagnosis of the APS

APS diagnosis is based on the presence of at least one clinical and one laboratory criteria; clinical and laboratory criteria are shown in Table 2. Epidemiologically, the APS is more frequently diagnosed among women in a proportion 4:1 and is more commonly found in young adults between 20-40 years although it may affect children and old people.[22–24]

1.3. Treatment of the APS

1.3.1. Anticoagulant treatment. Up to these days, standard treatment is anticoagulation with vitamin K antagonists (VKA) in patients who develop thrombosis.[2] Standard treatment of CAPS is based on high doses of steroids and heparins. For patients with obstetric manifestations, standard treatment also may include the administration of aspirin in combination with unfractionated heparin or low molecular weight heparin.[2] Recently, successful experiences for anticoagulation based on the use of direct oral anticoagulants namely rivaroxaban, apixaban, edoxaban, and dabigatran, has been published.[25]

Table 1. Clinical manifestations of APS

Frequent (>20% of the cases)

- Venous thromboembolism
- Thrombocytopenia
- Miscarriage or fetal loss
- Heart attack or transitory ischemic attack
- Migraine
- *Livedo reticularis*

Less frequent (10%-20% of the cases)
- Cardiac valve disease
- Pre-eclampsia o eclampsia
- Preterm birth
- Hemolytic anemia
- Coronary artery disease

Unusual (<10% of the cases)
- Epilepsy
- Dementia
- Chorea
- Retinal artery occlusion
- Pulmonary hypertension
- Venous leg ulcer
- Digital gangrene
- Osteonecrosis
- Nephropathy
- Mesenteric Ischemia

Rare (<1% of the cases)
- Adrenal bleeding
- Transverse Myelitis
- Budd-Chiari syndrome
- Sneddon syndrome
- Respiratory distress syndrome
- Addison syndrome
- Regenerative nodular hyperplasia of the liver
- Osteonecrosis
- Cutaneous necrosis

Modified from[21,97]

Table 2. Classification criteria to diagnostic APS

Clinical criteria

Vascular thrombosis
1. One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ confirmed by imaging studies or histopathology, thrombosis should be present with no evidence of inflammation in the vessel wall.

Pregnancy morbidity
1. One or more inexplicable deaths of a morphologically normal fetus at or beyond the tenth week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus or
2. One or more premature births of a morphologically normal neonate before the 34th week of gestation as results of eclampsia and severe preeclampsia, or recognized features of placental insufficiency or
3. Three or more inexplicable consecutive spontaneous abortions before the tenth week of gestation, with maternal anatomic or hormonal abnormalities, and paternal and maternal chromosomal causes excluded

Laboratory Criteria
A. LA present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis
B. aCL antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (>40 GPL or MPL units, or >99th percentile), on two or more occasions, at least 12 weeks apart, measured by standardized ELISA
C. α-β2GPI of IgG and/or IgM isotype in serum or plasma in titer >99th percentile, present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.

aCL: anticardiolipin antibody; α-β2GPI: anti-β-2-glycoprotein I antibody; LA: lupus anticoagulant. Modified from[54,118]

1.3.2. Alternative therapies. Several alternative therapies in addition to anticoagulant therapy such as statins and hydroxychloroquine (HQ) have been described. HQ has some effects *in vitro* that may help the management of APS, for example, reducing blood viscosity and platelet aggregation.[25] Also, it may have immunological effects such as inhibition of activation of intracellular Toll-like receptors (TLRs) as well as reduction of the production of IL-1, IL-2, IL-6, and TNF-α.[25] Moreover, HQ reduces the activation and expression of endosomal NADPH oxidase 2 (NOX2) in human umbilical vein endothelial cells (HUVECs) stimulated with TNFα or sera from preeclamptic women. Lastly, it prevents the loss of zonula occludens 1 (ZO-1) protein, thus, reducing the TNFα or preeclamptic sera-induced increased permeability of the HUVECs monolayer.[26,27]

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase that inhibit the synthesis of cholesterol and enhances its clearance by raising the level of low-density lipoprotein receptors.[28] It has been described that they may block some aPLs-induced effects. Indeed, some of these effects are: enhancing tissue plasminogen activator (tPA) expression while inhibiting the endothelin-1 (ET-1) expression (consequently, they may reduce the production of reactive oxygen species); inhibition of platelet adhesion and aggregation; inhibition of recruitment of inflammatory cells by repressing the expression of adhesion molecules (such as intercellular adhesion molecule 1 -ICAM-1- and P-selectin); reduction of the levels of C-reactive protein; shifting from T_H1 towards T_H2 differentiation; and inhibition of the upregulation of endothelial major histocompatibility complex class II and costimulatory molecules. All these effects could be dependent or independent of the reduction of cholesterol levels.[28,29] Furthermore, statins like mevastatin and pravastatin induce the expression of heme oxygenase (HO)-1 through the NrF2 signaling pathway[30] or the PKCa/Pyk2/p3 MAPK/ JNK1/2/AP-1 signaling pathway.[31] The expression of HO-1 inhibits the activation of NF-κB p65 (31) and additionally, activation of NrF2 which allows the transcription of antioxidant proteins such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).[30] As pravastatin does, SM934 an artemisinin derivative, induces the translocation of NrF2 to the nucleus where it stimulates the transcription of HO-1, SOD, GPx, and CAT.[32]

2. Structure and Physiological Roles of beta-2-Glycoprotein 1 (β2GP1)

β2GP1 is the main target of autoantibodies in APS. This protein, also known as apolipoprotein H, is a heavily glycosylated plasmatic glycoprotein which is found in plasma in a freeform or in complex with lipoproteins (30%).[33] β2GP1 is a single chain glycoprotein composed of 326 amino acids with a molecular weight between 42 to 50 KDa that is synthesized by endothelial cells, hepatocytes, and trophoblast cells. Plasma levels range between 50-400 μg/mL.[29,34–38]

β2GP1 is constituted by five domains (DI-DV); the fifth domain has a positive zone composed of 14 lysines which serve as an anchor for anionic phospholipids (AP). β2GP1 is cleaved by plasmin or activated factor X (FXa) between Lys317-Thr318 in the fifth domain resulting in the formation of nicked β2GPI (nβ2GPI) which has the ability to bind plasminogen; this effect decreases the generation of plasmin in the presence of tPA and fibrin.[22,34,39,40]

In solution, β2GP1 has three different conformations: a. A circular or closed form in which domain I (DI) interacts with domain V (DV). While in this form, the B cell epitope is hidden from the immune system and binds less efficiently to AP and complement; b. After binding to anionic surfaces such as cardiolipins through DV or in presence of anti-β2GP1, the protein changes its conformation to an open variant called J form and, in this conformation, DI epitopes are exposed to the environment allowing the binding of antibodies. c. Between the open and close forms there is an intermedium S form which occurs when β2GP1 interacts with lipopolysaccharides (LPS) through the C-terminal domain (a fact that, potentially, works as an LPS scavenger).[22,34,35,41,42] Using electron microscopy, it was demonstrated that >99% of plasma β2GP1 is in a closed conformation and that, after interacting with AP, changes its conformation to an open form.[22,42] β2GP1 may bind to several biomolecules like phospholipids, sulpholipids, LPS, heparin, C3 component of the complement system, factor XII (FXII), annexin A2, tPA, platelet glycoprotein 1b (GP1b), megaline, platelet factor 4 (PF4), plasminogen, fibrin, and von Willebrand factor (VWF).[34,43–46] Therefore, a myriad of functions for β2GP1 on the blood coagulation system, immunity, and angiogenesis have been described.

2.1. β2GP1 as a Modulator of Hemostasis

β2GP1 inhibits the FXII-dependent activation of fibrinolysis.[45,47] Also, it may have a procoagulant function because, after binding to thrombin, it avoids its inactivation by heparin cofactor II; moreover, β2GPI inhibits the inactivation of activated factor V (aFV) by activated protein C (aPC).[22,34] Furthermore, β2GP1 has antiplatelet effects because it decreases platelet adhesion by inhibiting the interaction between platelets and VWF in active conformation. While β2GP1 does not affect adenosine diphosphate (ADP) or collagen-induced platelet aggregation, it may affect ristocetin-induced platelet aggregation. In summary, β2GP1 has anti-adhesive and anti-aggregation properties.[48] Finally, β2GP1 is profibrinolytic because it binds to tPA acting as a cofactor for the generation of plasmin. However, the β2GP1-plasminogen complex inhibits the generation of plasmin.[39,40,43,46]

2.2. β2GP1 as an Opsonin

β2GP1 binds to phosphatidylserine-containing vesicle-like apoptotic bodies or microvesicles, enhancing their clearance by interacting with TLR2, TLR4, annexin 2, and apolipoprotein E receptor 2 (ApoER2).[22,34]

2.3. β2GP1 as a Scavenger of LPS

β2GP1 interacts with LPS through the fifth domain; this interaction causes a conformational change of β2GP1 towards the

J conformation. This change reduces the tissue factor (TF) expression on endothelial cells and monocytes after being stimulated with β2GP1-LPS complex as compared with monocytes and endothelial cells stimulated with only LPS.

2.4. *β2GP1 as a Modulator of the Complement System*

β2GP1 inhibits the activation of the classic and alternative pathways of the complement system because it inhibits the generation of C3-convertase complex[22] and the subsequent generation of C5 and the terminal complement complex C5b-9.

2.5. *β2GP1 as a Modulator of Angiogenesis*

β2GP1 and nβ2GP1 do not have effects on the proliferation of human aortic endothelial cells and HUVECs in culture, however, in the presence of vascular endothelial growth factor (VEGF), nβ2GP1 blocks the proliferation of HUVECs. Both forms of β2GP1 at concentrations between 1-4μM inhibit the migration and tube formation of HUVECs;[39] this anti-migratory effect is reversed by the addition of anti-β2GP1.[49,50] As a consequence, β2GPI may be considered as an angiogenesis inhibitor both, *in vivo* and *in vitro*. On the other hand, while angiostatin (AS4.5), a plasminogen fragment, inhibits the proliferation, migration, and tube formation of HUVECs, such effects may be reversed by addition of nβ2GP1.[39] nβ2GPI has dual effects in presence of AS4.5; at low concentrations, it functions as AS4.5 inhibitor, however, at higher concentrations it works as an angiogenesis inhibitor.[39] These effects depend on the inhibition of the phosphorylation of ERk1/2, AKT, and endothelial nitric oxide synthase (eNOS) induced by the treatment with VEGF.[49,51] Figure 1 shows a summary of the β2GP1 functions.

3. Antiphospholipid Antibodies (APLs)

3.1. *Heterogeneity and Diagnostic Significance of the Different APLs*

aPLs antibodies are the etiological mediators for the thrombotic and obstetric manifestations of the APS. aPLs are a heterogeneous family of auto-antibodies that recognize plasmatic proteins with high affinity for AP-binding proteins, namely β2GP1 and blood coagulation factor II (prothrombin).[5,42,52,53]

Diagnostic criteria for APS include the persistence of LA, aCL, and/or α-β2GP1 at medium to high titers in the plasma of the patient.[3,5,41] These antibodies included in Sydney's classification are aCL (IgM/IgG), α-β2GP1 (IgM/IgG), and the LA.[54]

In 2006, the prevalence of IgG subclasses in 74 APS patients was analyzed and it was found that the predominant subclasses in aCLs were IgG1, followed by IgG2, IgG3, and IgG4. Moreover, analysis of the IgG subclasses of α-β2GP1 found that the predominant subclasses were IgG2, IgG1, IgG3, and IgG4.[55] In 2005, the IgG subclasses found in 29 patients with

persistent titers of α-β2GP1 were IgG2, IgG3, IgG1, and IgG4.[56] In 2018, IgG subclasses for α-β2GP1 and anti-domain 1 of β2GP1 antibodies (α-DIβ2GPI) were evaluated in 19 APS patients of which 18/19 had α-DIβ2GPI and, contrasting with previous reports, for α-β2GP1 the predominant subclasses were IgG1, IgG3, IgG2, and IgG4. In contrast, for α-DIβ2GPI the predominant subclasses were IgG1, IgG3, IgG2, and IgG4.[57] In all of the cases, the authors reported a predominance of IgG1 and IgG2 for α-β2GP1, IgG1 for aCL, and IgG3 for α-DIβ2GPI. The importance of the antibody subclass relies on their biological functions; all subclasses activate complement which plays a fundamental role in the physiopathology of this disease. Table 3 listed the different antibody specificity described in APS patients.

From 2004 to 2010, using twelve monoclonal aPLs (maPLs) from four APS patients, the size and length of the hemostatic plug, the ability to activate or inactive serine proteases, and the cross-reactivity by functional assays, were analyzed. Authors found a high rate of cross-reactivity between the maPLs and concluded that this high cross-reactivity to several serine proteases could be due to the recognition of shared epitopes of their catalytic domain while those antibodies recognize serine proteases and β2GP1 may share epitopes.[58-62] They assumed that just only one antibody could be the cause of multiple pathological processes.

Infections may induce transient aPLs however, according to the Sapporo classification, these autoantibodies are not associated with a higher thrombotic risk.[23]

3.2. *Association Between APLs and Thrombosis*

The presence of LA is strongly associated with a higher risk of venous and arterial thrombosis as compared with the risk imposed by aCL and α-β2GP1. Patients with positive to one or more aPLs (LA, aCL, and/ or α-β2GP1) have a higher risk of developing thrombosis as compared with those with positivity to only one aPLs however, triple-positive patients have the highest risk for thrombosis as well as pregnancy complications. As compared with healthy subjects, the cumulative incidence of thrombotic events is 12.2%, 26.1%, and 44.2% after 1, 5, or 10 years follow up, respectively. Triple positivity is a risk factor of pregnancy complications in patients with primary APS. It is likely that α-DIβ2GP1 imprints a higher risk for thrombosis and pregnancy morbidity as compared with other anti-β2GP1 subpopulations.[2,52]

4. Pathogenetic Mechanisms in APS

4.1. *Obstetrical APS*

4.1.1. Placental histopathology. In obstetrical APS, a failure in the spiral artery remodeling by the extravillous trophoblast induces a reduction or interruption of the maternal blood supply to the placenta generating either hypoxic/ ischemic damage or inadequate nutrient influx to the fetus and/or an increased flux that may cause placental damage.[63]
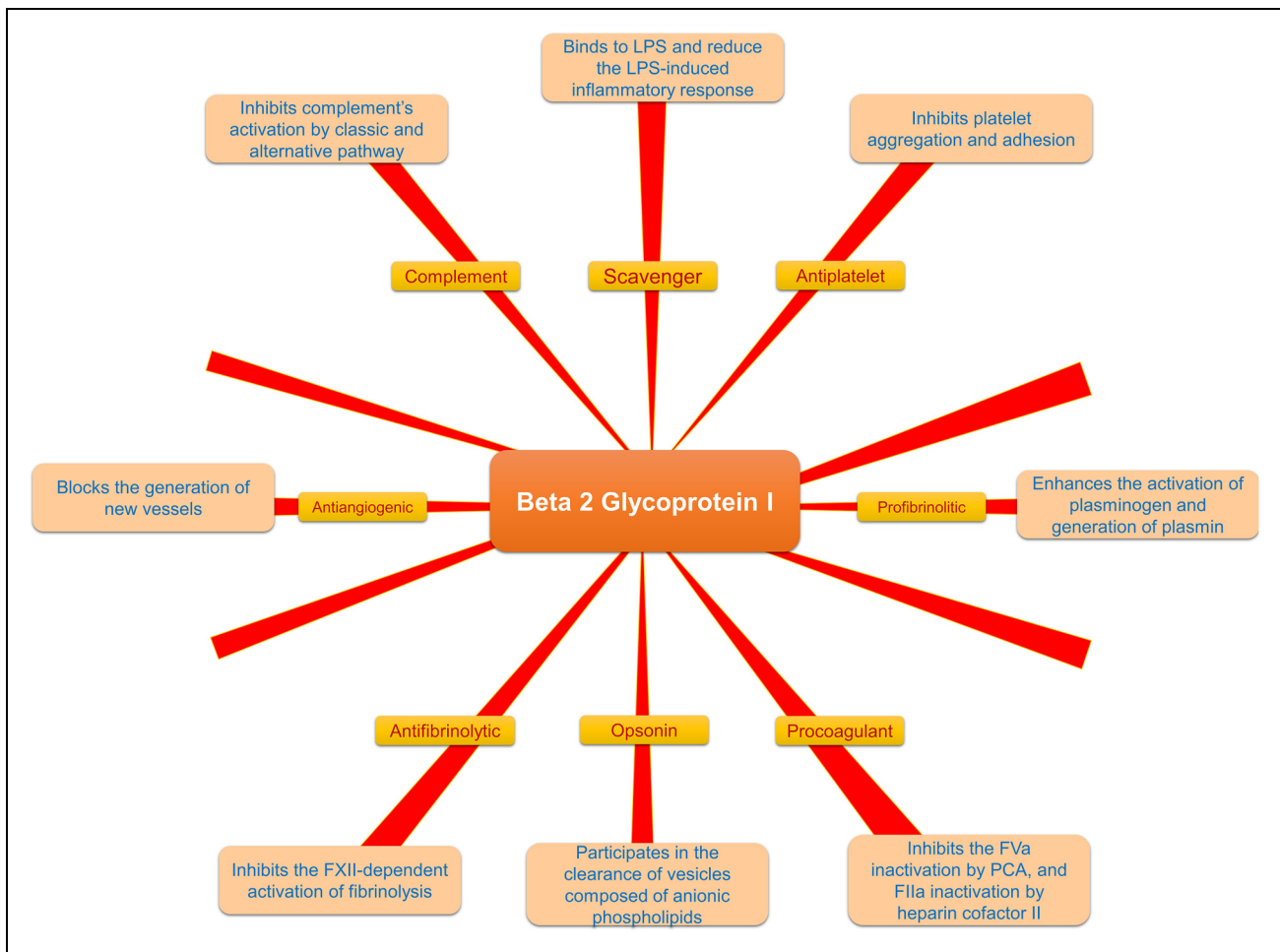
Figure 1. Physiological roles of β2GPI. β2GPI has several functions. It may act as an opsonin leading the clearance of anionic phospholipid composed vesicles as well as a scavenger of LPS. These two effects reduce the inflammatory response. On the other hand, β2GPI is a modulator of the complement system, angiogenesis, and hemostasis.

In aPLs-treated mice, the histopathological findings show a higher TF staining on the decidua and embryonic debris as compared with mice tissues treated with IgG from healthy subjects (HS). This increased TF was neither associated with a higher staining for fibrin nor evidence of thrombosis, a fact that strongly suggests that the TF-dependent thrombosis induced by aPLs is not the cause of fetal loss. Fetal loss, C3d deposition, and infiltration of neutrophils induced by aPLs were inhibited by inhibiting TF with specific antibodies.[64] On the other hand, as compared with the placental of healthy women, the histopathological findings in the placenta of APS patients with obstetric manifestations were a greater decidual, vascular and intervillous necrosis; increased syncytial nodes; abnormalities in spiral artery remodeling; reduction of vasculo-syncytial membranes; placental infarction; fibrin deposition in syncytial nodes; and stromal fibrosis.[63,65]

Because β2GP1 is constitutively expressed on the surface of all subpopulations of the placental trophoblast and maternal endothelial cells, it is likely to hypothesize that this protein is the target for α-β2GP1 and other aPLs. Binding of these autoantibodies to β2GP1 may induce a proinflammatory, antimigratory, and antiangiogenic phenotype in the trophoblast.[63]

4.1.2. Complement activation. Plasma levels of C5a and the complement terminal complex C5b-9 were analyzed in 60 APS patients (43 non-pregnant women and seventeen pregnant women) and sixteen healthy women (eight non-pregnant women and eight pregnant women). Patients were classified according to their thrombotic and/or pregnancy morbidity. Moreover, pregnant patients were further classified based on the pregnancy prognosis as favorable or unfavorable. Levels of C5a in patients with thrombotic manifestations without a history of pregnancy morbidity were similar to those with healthy women. In contrast, C5a levels were higher in patients with thrombotic manifestations and pregnancy morbidity history as compared with healthy women. Furthermore, C5a levels in patients with obstetrical manifestations but favorable prognosis were not different from the levels of controls. In contrast, C5a levels in pregnant patients with unfavorable prognosis were higher than those of control women. Moreover, levels of complement terminal complex C5b-9 and complement

Table 3. Antibodies specificity in APS patients

Antigen / antigen complex
Phospholipids
• Anionic phospholipids (Cardiolipins, phosphatidylserine)
• Phosphatidylethanolamine
Components of the coagulation and fibrinolysis network
• β–2-Glycoprotein I
• Prothrombin
• Thrombin
• Antithrombin/ thrombin complex
• High molecular and low molecular weight kininogen
• Prekallikrein
• Protein Z
• Protein C
• Protein S
• Thrombomodulin
• Plasminogen tissue activator
• Annexin II
• Annexin V
• Activated factor IX
• Factor X
• Factor XII
• Heparin
• Tissue factor pathway inhibitor
• Lipoprotein
• Oxidized low-density lipoprotein
• High-density lipoprotein
• Apolipoprotein A-1

Modified from[53,119]

regulatory proteins (CD46, CD55, and C59) were analyzed in APS and their controls. In APS patients, there was an increase in the C5b-9 levels and a reduced expression of CD59. Additionally, in pregnant patients with unfavorable prognosis, increased C5b-9 levels and reduced CD55 levels were observed. As a consequence, it is likely that a reduced expression of the complement regulatory proteins may promote a hyperactivation of the complement system and the subsequent induction of inflammation, thrombosis, and tissue damage.[66]

4.1.3. Neutrophil activation mediated by aPLs.
The effects of the neutrophil's extracellular traps (NETs) on activation of HUVECs and trophoblast have been analyzed. Increased levels of myeloperoxidase (MPO), cell-free DNA, MPO-DNA complex, and neutrophil elastase (NE) were found in the serum of 22 patients with obstetric APS as compared with controls. When the ability to release NETs in APS patients versus controls was evaluated, it was found that neutrophils from APS patients released spontaneously more NETs than their counterparts. This effect was attributed to aPLs of the APS patients because incubation of neutrophils from healthy women with aPLs induced an enhanced release of NETs secondary to increased production of NADPH oxidase-dependent reactive oxygen species (ROS).[67] On the other hand, the anti-migratory and anti-angiogenic ability of NETs on trophoblast cells and endothelial cells have been studied.[67] In murine models, neutrophils may be directly activated by aPLs through binding of

β2GP1 or, indirectly, through PLs-induced activation of the complement system and generation of C5a which, in turn, interacts with C5aR on the neutrophil's surface or forms the TF/activated factor VII/ protease-activated receptor 2 (PAR2) complex. Main effects of neutrophil activation by aPLs were chemotaxis, NETs release, enhanced phagocytic capacity, increased intracellular calcium concentration, degranulation, decidual neutrophil infiltration, enhanced TF expression, and increased generation of ROS.[2,6]

4.1.4. Role of oxidative stress in obstetrical APS.
Oxidative stress is defined as a disequilibrium between the production of reactive species and antioxidant defenses leading to abnormalities in signaling pathways and potential tissue damage.[68,69] Oxidative stress has been implicated in numerous pathologies like neurodegenerative, cardiovascular[70–72] or autoimmune disease (systemic lupus erythematosus or rheumatoid arthritis)[73–75] Several authors have implicated oxidative stress in the physiopathology of the APS. In placental cultures, it was found that interaction between maPLs and placental explant induces ROS production; furthermore, these maPLs could bound in a dose-dependent manner to mitochondria isolated from placenta. The meaning of this observation is that target antigens from aPLs are present in the mitochondria and this interaction induces and enhances the respiration rate raising ROS production by mitochondrial complex I, II, and mGDPH.[76]

Activation of trophoblast by aPLs, proinflammatory cytokines, TLRs ligands such as histones and NE, causes oxidative stress, lipoperoxidation, production of proinflammatory cytokines and chemokines (mainly IL-8) by the trophoblast, induces an increased expression of prostaglandin F2 alpha-receptor and oxytocin receptor as well as an increased production of prostaglandins by cyclooxygenase-2. All these events may cause myometrial contraction, cervical ripening, membrane rupture, and uterine contractions that result in the induction of labor or fetal death.[64,77–79]

In conclusion, in obstetric APS patients, aPLs binds to β2GP1 in the trophoblast surface causing its activation and the consequent production of proinflammatory cytokines and chemokines that recruits neutrophils. These cells may be either directly activated through the binding of aPLs to β2GP1 or indirectly, by complement activation products induced by aPLs binding. Their activation would raise because of autocrine mechanisms that may induce TF expression and its binding to factor VII, a complex that generates down-stream signaling through PAR2. All these activation events produce chemotaxis in the decidua, increase phagocytic capacity, enhance the production of pro-inflammatory cytokines, augment of NETs release (a positive feed-back mechanism), and increase the activation and generation of proinflammatory cytokines resulting in premature birth, fetal death or miscarriage, the characteristic findings of obstetric APS. Figure 2 shows a summary of the pathophysiological mechanism of obstetric APS.

4.2. Thrombotic APS

Several mechanisms explain the thrombotic events in APS patients. For this review, we grouped them into four sections including platelet, endothelial, eosinophil and soluble factor activation.

4.2.1. Role of oxidative stress in thrombotic APS.

In order to induce aPLs, in a murine model with severe combined immunodeficiency injection of hybridomas generated a reduction of paraoxonase activity, total antioxidant capacity, production of NO as well as the expression of induced oxide nitric synthase (iNOS). On the contrary, an enhanced production of nitrotyrosines was found when hybridomas secreting antibodies with no aPLs specificity were administered.[80]

In APS patients, a higher concentration of inflammatory markers such as C reactive protein, serum amyloid A, and PGE_2 has been described. Also, inflammatory cytokines and chemokines such as VEGF, IL-8, MIP1α, and tPA as well as markers of oxidative stress like 8-isoprostane and NO are elevated as compared with heathy individuals.[81,82] The augment of 8-isoprostane and PGE_2 was more pronounced in triple-positive patients was found in single and double-positive APS patients.[81] In monocytes or neutrophils from APS patients, a higher activity and expression of TF,[81,82] PAR2, and FLT1 has been described.[82] Moreover, as compared with healthy individuals, a higher production of superoxide and reduced concentrations of glutathione, reduced catalase, and glutathione peroxidase activity have been described. Also, as compared with controls, these reductions were associated with a reduced nuclear abundance of Nrf2 in APS patients.[82] However, in the study by Vaz *et al* no differences were found between thrombotic PAPS patients (n = 70) and controls (n = 74) when comparing total antioxidant capacity, oxidative damage and oxidative balance measured by the concentration of protein carbonyl and 8-isoprostane.[83] We highlight that the aforementioned studies[81,82] did not include a homogeneous group of PAPS patients as was done in the study by Vaz *et al*[83] The study by Sciascia *et al*[81] classified the APS patients (n = 45) as PAPS or APS associated with other immunological diseases, and the study by Perez-Sanchez *et al*[82] enrolled 25 APS patients with thrombotic manifestation and 19 with pregnancy morbidity. In addition, the different thrombotic treatments in patients may explain the discrepancies between studies. All APS patients in the study by Vaz *et al* were on warfarin treatment, while patients in the study by Perez-Sanchez *et al*[82] were under treatment with warfarin or acenocoumarol, ASA or clopidogrel, or hydroxychloroquine.

On the other hand, to evaluate whether aPLs have a causal role on the APS patients, monocytes were incubated with a pool of IgG from APS patients (IgG-APS) or healthy subjects (IgG-HS). Monocyte stimulated with IgG-APS showed a higher activity and expression of TF, an enhanced superoxide production, a reduction of GSH levels, and a decrement of the nuclear abundance of Nrf2 as compared with monocytes stimulated with IgG-HS.[82,84] These findings strongly suggest that aPLs have a causative role on the characteristic effects observed in APS patients.

In order to assess if supplementation with antioxidants would influence the concentration of inflammatory molecules and the antioxidant capacity, monocytes were treated with antioxidant before stimulation with aPLs. Incubation with antioxidants reduced the superoxide production and TF expression induced by IgG-APS. Moreover, increased levels of GSH, production of NO, and expression of iNOs were also observed. Moreover, antioxidant treatment reduced the membrane expression of TF, VEGF, and Flt2; probably, this was a consequence of a reduced activity of the p38 MAPK and NF-kb signaling pathways.[82,84] These *in vitro* effects were also demonstrated in APS patients supplemented with vitamin C or E in whom reduced urinary isoprostane levels and concentration and activity of in monocytes were found.[84]

The above mentioned information may suggest that elevated levels of oxidant stress markers found by some authors, and the proinflammatory /procoagulant phenotype were, at least partially induced by the oxidative stress induced by aPLs. The discrepancies found in the levels of oxidative stress markers measured in APS patients should be addressed in further studies.

4.2.2. Platelet activation mediated by aPLs.

Several platelet activation mechanisms induced by α-β2GP1 have been described. For example, α-β2GP1binding to PF4 stored in alpha granules induces a conformation change that potentiates its immunogenicity.[44] Platelets exposed to the α-β2GP1-β2GP1-PF4 complex increases the generation of thromboxane A_2 (TxA_2) as compared with platelets stimulated with the α-β2GP1-bovine serum albumin-PF4 complex. This increased platelet activation was mediated by P38MAPK phosphorylation.[44] Another described mechanism is a direct interaction of α-β2GP1 with β2GP1 which induces dimerization of β2GP1 as well as a 100-fold increase of its phospholipid-affinity.[85] The interaction between dimeric β2GP1 with platelets is mediated by the ApoER2'-glycoprotein (GP) Ibα complex, a fusion event secondary to the DI-DIII and DV domains from β2GP1 and the binding site of VWF in GPIbα. Generation of this protein complex results in a higher platelet sensibility for a second stimulus such as the exposition of collagen upon vascular damage. These phenomena are mediated by MAP kinase pathway which triggers the production of TxA_2.[85,86]

In searching for the role of platelets on endothelial activation and fibrin generation caused by α-β2GP1, a similar expression of ICAM-1 in the presence or absence of α-β2GP1 was found, nonetheless, in the presence of platelets and α-β2GP1, expression of ICAM-1 and fibrin generation was boosted by 20-500-fold. It was shown that α-β2GP1 did not bind to endothelial cells but to platelets suggesting that first, platelet thrombus promotes the endothelial activation upon vascular damage in presence of α-β2GP1 and second, in the presence of α-β2GP1 recruitment and activation of platelets is increased.[87] Likely explanations for these findings are an increased expression of adhesion molecules like ICAM-1[87] or VWF dysfunction. Indeed, VWF has both, a higher half-life in APS patients
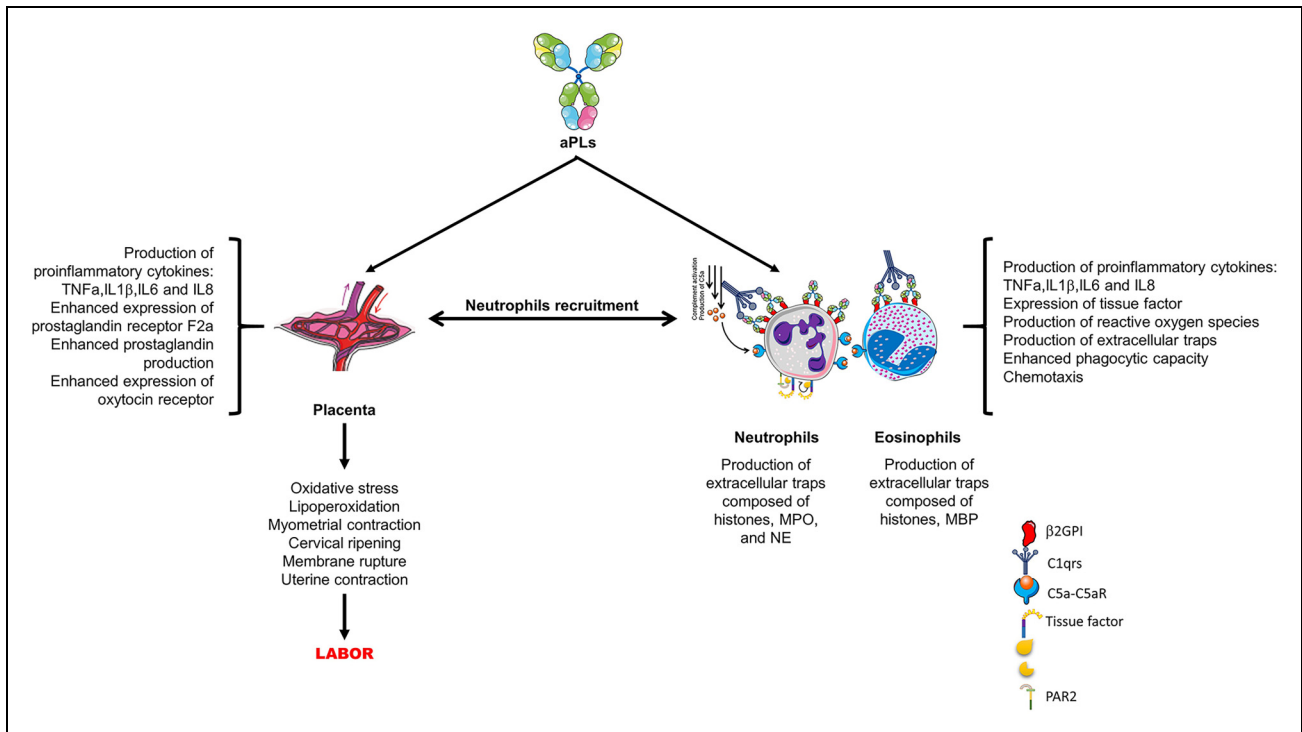
Figure 2. Pathophysiology of obstetric APS. aPLs may directly activate the placenta through the interaction of anti-β2GPI with β2GPI and/or annexin A2, leading to the production of pro-inflammatory cytokines and chemokines (primarily IL-8) which may recruit neutrophils. In turn, these cells are activated by aPLs which may induce the generation of NETs and the consequent activation of the placenta by the coupling to TLR2/4. Finally, activation and lipid peroxidation of the placenta may trigger myometrial and uterine contraction, cervical ripening, membrane rupture, and labor induction.

as compared with controls as well as an abnormal VWF multimeric pattern which is characterized by larger size multimers and increased concentration of ultra-large multimers (ULVWF). An explanation for these facts may be a decreased activity of the metalloproteinase with a thrombospondin type 1 motif member 13 (ADAMTS13) found in APS patients which is likely secondary to anti-ADAMTS13 antibodies.[88] Another explanation for the presence of larger size and high concentrations of ULFVW is the high apoptosis indexes observed in APS patients which increases the availability of vimentin to bind to A2 domain of VWF.[89] As a consequence, vimentin may compete with ADAMTS13 for the binding site on VWF. Another mechanism that increases the vimentin availability is the aPLs-induced enhanced platelet, endothelial, monocyte, and neutrophil activation, as described in the text.

An increased concentration of thrombospondin 1 (TSP1) is another mechanism that explains by which the size and distribution of ULFVW is modified in APS patients. TSP1, a 450 KDa homotrimeric glycoprotein, is synthetized by smooth muscle cells, fibroblasts, megakaryocytes/platelets, and endothelial cells. TSP-1 is stored in alpha granules and Weibel Pallade bodies (WPB), in platelets and endothelial cells, respectively. TSP1 plasma concentration is 0.1-0.3 µg/mL and it may interact with multiple receptors such as CD36, integrins, CD47, the GPIb/IX/V complex, heparan sulfate, VWF, fibrinogen, laminin, fibronectin, and collagen.[90] TSP1 anchors the blood

clot in damaged vessels preventing embolization of the clot. Also, it binds to the A3 domain of VWF generating a competitive interaction with ADAMTS13 and decelerating its proteolytic action on VWF.[90]

In APS patients, TSP1 concentration is higher than in controls.[91] Furthermore, higher concentrations of TSP-1 in APS patients with arterial or venous thrombosis as compared with patients with obstetric APS have been found. TSP1 concentrations are higher in patients with acute thrombotic events versus patients with a history of thrombosis. Elevated plasma and platelet TSP1 are likely induced by the α-β2GP1-β2GPI-PF4 complex which induces release of the stored protein from platelet alpha granules and endothelial WBPs.[91] Therefore, raised ULFVW concentrations are due to a decreased ADAMTS13 activity and increased availability of vimentin and TSP1 in response to enhanced platelet, endothelial cell, and monocyte activity as well as to the presence of α-β2GP1 which reduces de binding of β2GP1 to active VWF forms.

Another mechanism that may trigger platelet and leukocyte activation in APS patients is the presence of anti-vimentin/cardiolipin antibodies. CL, a lipid of the inner mitochondrial membrane, is redistributed to the plasmatic membrane after induction of apoptosis mediated by FAS receptors and/or TNF-α receptor.[92] The redistribution of CL depends on vimentin, a cytoskeletal protein expressed on mesenchymal derived cells such as neutrophils, T cells, activated macrophages,

endothelial cells, smooth muscle cells, activated platelets, and apoptotic cells[43,92,93] As β2GP1, vimentin interacts with CL and, as a consequence, it may be a cofactor for the presentation of CL to the immune system increasing its immunogenicity.[92,94] In patients with seronegative APS (n = 29), 55.2% had anti-vimentin/CL antibodies as was also observed in APS patients with positive aCL titers in whom 92.5% had anti-vimentin/CL antibodies.[94] In both studies, the presence of anti-vimentin/CL antibodies was not related to thrombotic events. Despite the lack of association between thrombosis and the presence of anti-vimentin/CL antibodies, *in vitro* studies strongly suggest an effect of these antibodies on hemostasis. For example, stimulation of HUVECs with anti-vimentin/CL antibodies induced phosphorylation of the interleukin-1 receptor-associated kinase (IRAK) and activation of the nuclear factor-kappa B (NF-κb), effects that were not observed after stimulation with IgG in controls.[94] Moreover, incubation of whole blood with IgM anti-vimentin antibodies (AVA) caused platelet depletion, formation of platelet-leukocyte aggregates, expression of CD62P on the platelet surface, expression of TF on the leukocyte surface, and fibrin and C3d deposit on the platelet and leukocyte membrane. All these effects were inhibited by adding recombinant vimentin.[93] AVA bind preferentially to neutrophils, monocytes, and activated platelets however, platelet activation is due to the release of inflammatory mediators such as platelets-activating factor (PAF) by leukocytes but not due to a direct effect of AVA,[93] as shown after adding PAF inhibitor to the whole blood.

The origin of AVA has not elucidated however, it is proposed that induction of these antibodies in autoimmune diseases results from an excessive exposition of vimentin to apoptotic cells. Although cleavage of vimentin by caspases is a requisite for apoptosis,[93] evidence regarding this dysregulation process of apoptosis in APS patients has not been proven.[95]

Finally, platelets are also activated by NETs, specifically by histones. These proteins activate platelets expressing phosphatidylserine, P-selectin, and FVa on their surface. This activation depends on the presence of ADP, P2Y12 receptor, and GPIIb/IIIa.[96]

4.2.3. Endothelial activation mediated by aPLs. α-β2GP1 binds to endothelial cells in the presence of platelets however,[87] other authors claim that binding of α-β2GP1 to endothelial

Table 4. Receptors involved in the recognition of aPLs

Cell type	Receptors
Endothelium	Annexin A2, ApoER2'', β2GPI,TLR2/4
Monocytes	Annexin A2 TLR2/4
Platelets	GPIbα, ApoER2'
Neutrophils	β2GPI, TLR2/4
Trophoblast	Annexin A2 TLR2/4
Decidual cells	Annexin A2 TLR2/4

ApoER2′: apolipoprotein E receptor 2′; β2GPI: β-2-glycoprotein I; GPIbα: glycoprotein Ibα; TLR2/4: Toll-like receptor 2/4.

cells is through annexin A2 (A2).[97–99] A2 is a profibrinolytic receptor that binds to plasminogen and tPA, acting as a cofactor for plasmin generation and allowing the localization of fibrinolysis to the endothelial cell surface.[97–99] A direct activation of endothelial cells by anti-A2 and/or through the complex α-β2GP1-β2GPI-A2 has been demonstrated.[98] Endothelial cell activation results in an increased expression of E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and TF.[99] APS patients who had anti-A2 have a 35-83% reduction of tPA-dependent plasmin production. The high variability of this reduction was probably secondary to a specific epitope on A2, the presence of multiple epitopes, and/or the presence of an alternative endothelial plasminogen receptor.[97]

β2GP1 binds tPA with high affinity, increasing 19.7-fold the plasmin generation by tPA as compared with A2 binding which increases 63-fold. The role of β2GP1 on thrombus dissolution has been evaluated. In the absence of β2GP1, dissolution kinetics was reduced and reversed when β2GP1 was added. Evaluating the effect of α-β2GP1 on tPA activity, the IgG fraction of APS patients inhibited the tPA-dependent plasminogen activation in a concentration-dependent way.[43] Thus, β2GP1 and A2 are tPA synergic cofactors for plasminogen activation and, as a consequence, antibodies against them reduce plasminogen activation predisposing to thrombosis in APS patients.

Another mechanism for β2GP1-induced endothelial cell activation has been suggested. α-β2GP1 cross-recognize β2GP1 as plasminogen on the surface of endothelial cells inducing overexpression of ICAM-1 and subexpression of thrombomodulin (TM), a protein with a critical role in the protein C system, the most important natural anticoagulant mechanism.[46] Besides, other authors have shown that α-β2GP1 also induces the generation of ROS through the activation of MAP kinases.[100] The increased production of ROS triggers the production of nitric oxide (NO) by eNOS and, as a consequence, an increased production of ROS which consumes NO and decreases its release from endothelial cells,.[101] As a result, there is a decreased bioavailability of NO. On the other hand, ET-1 concentrations were analyzed in 27 APS patients, 11 and 16 with a history of venous or arterial thrombosis, respectively. Higher ET-1 concentrations were found in only eight out of sixteen patients with arterial thrombosis but no differences were found in patients with venous thrombosis. Moreover, aCL induced the production of preproendothelin-1 mRNA in presence of β2GP1.[102] In 2015, factors inducing ET-1 production were described including thrombin, ROS, and proinflammatory cytokines such as IL-1, IL-6, and TNFα.[103] All of these proteins are elevated in APS patients. Therefore, all these above mentioned facts strongly suggest that APS patients have abnormal endothelial vasomotor activity due to both, a reduced bioavailability of NO caused by excessive production of ROS and an increased production of ET-1.

Considering that endothelial activation by aPLs increases the expression of adhesion molecules such as P-selectin, ICAM-1, and VCAM-1 and that it induces TF overexpression and subexpression of TM, the induction of proinflammatory cytokines, high concentration of ET-1, low NO bioavailability, and
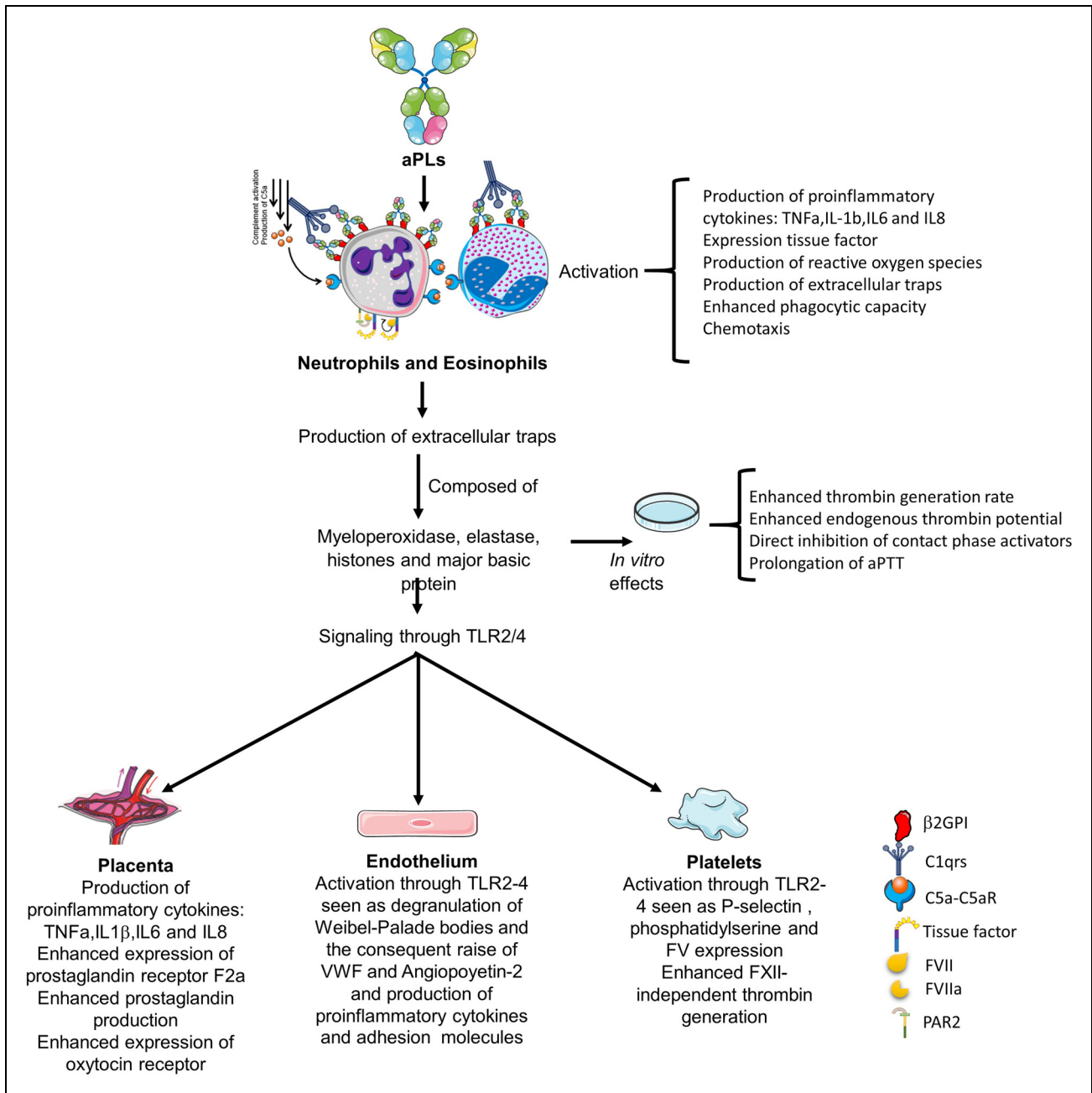
Figure 3. Histones can activate cells through the engagement of TLR2/4. aPLs may trigger the release of NETs composed mainly of histones, neutrophil elastase, and myeloperoxidase. These biochemical compounds are recognized by TLR2/4 and, when complexed, they may trigger the activation of the placenta, endothelium, and platelets, among other cells. Furthermore, these constituents may prolong the activated thromboplastin time test through the direct inhibition of the contact phase activation. In addition, they can enhance the thrombin rate generation and the endogenous thrombin potential.

higher ROS production, results in a pro-adhesive, pro-coagulant, pro-inflammatory, and pro-aggregatory endothelium. Table 4 shows the receptors involved in the recognition of aPLs on the cell surface of different cell types including endothelial cells.

On the other hand, histones can also activate endothelial cells. Stimulation of blood outgrowth endothelial cells with histones induce exocytosis of WPB and a subsequent rise in the concentration of VWF and angiopoietin 2 as well as an increased platelet-endothelium interaction through VWF. The exact activation mechanism induced by histones is not clear yet[104] however, it has been proposed that endothelial activation is mediated by TLR4.[105] Finally, studies in mice demonstrated that infusion of histones induces thrombocytopenia and increased VWF; this decrease of the platelet count was not related to VWF because the degree of thrombocytopenia was
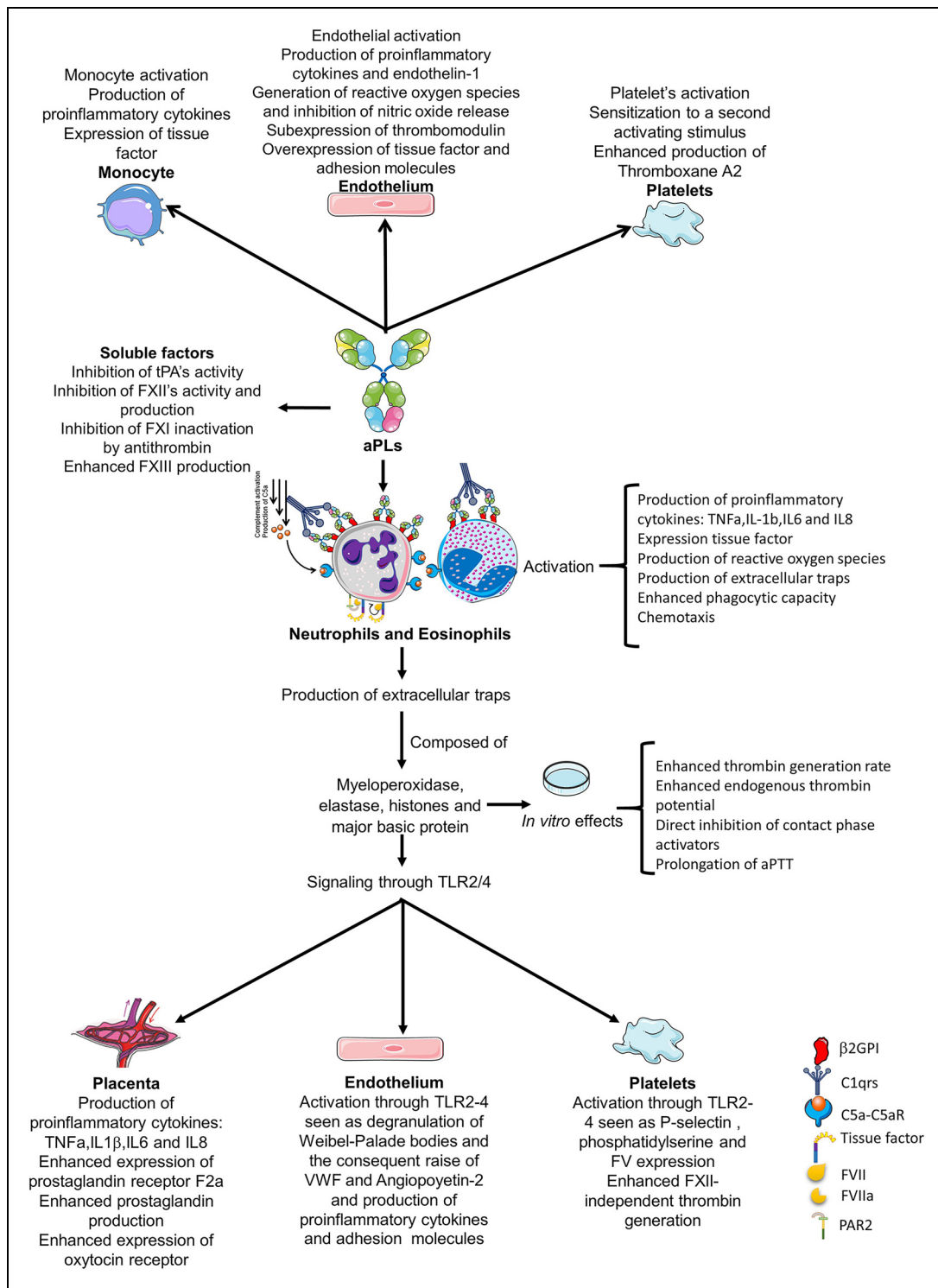
Figure 4. Physiopathology of APS. aPls may activate different cells. In monocytes, aPLs binds to β2GPI triggering the production of TF, cytokines, and proinflammatory chemokines. In endothelial cells, aPLs induce the production of chemokines, proinflammatory cytokines, TF, ROS, and ET-1 and the consumption of NO. On the other hand, aPLs increase the platelet sensitivity to its agonists while increasing the production of TxA2. The effects of aPLs on hemostasis and fibrinolysis range from inhibition of tPA activity and inhibition of FXII-mediated fibrinolysis to inhibition of FXI inactivation by antithrombin as well as increased FXIII synthesis. Indirectly, aPLs also activate cells involved in APS through the activation of neutrophils and the release of NETs. In NETs, DNA and elastases are recognized by TLR2/4, leading to activation of the placenta, endothelium, and platelets.

not different between histone-treated VWF deficient mice and histone-treated wild type mice.[104]

4.2.4. Eosinophilic granulocyte activation mediated by aPLs.

In 2018, a working group compared the protein composition of fibrin clots in controls, patients with deep venous thrombosis, and APS patients using liquid chromatography and mass spectrometry. Clots from controls and patients with deep vein thrombosis had higher levels of complement proteins such as C5-C9, platelet glycoproteins (GPIIb, GPIIIa), TSP1, neutrophil myeloperoxidase, histones (H2A/H2B), and major basic protein from eosinophils as compared with clots from APS patients.[1] These findings may reflect the involvement of extracellular traps in the development of thrombosis. One year later, another group showed the role of eosinophils on the development and stability of blood clots. They showed that clots of eosinophil-deficient mice were less stable in comparison to clots from wild-type mice. Furthermore, they demonstrated that platelet-eosinophil interactions increased platelet aggregation and triggered the production of eosinophil extracellular traps (EETs) which are differ from NETs because of their major basic protein (MBP) content. This protein has effects on clot stability because MBP-deficient mice had fewer stable clots and it has been shown that MBP binds to TM inducing an inhibitory effect on PC activation. Finally, MBP promotes platelet aggregation and thrombus development.[106]

4.2.5. Activation of plasmatic hemostasis.

The effect of β2GP1 on the activation of blood coagulation FXII induced by ellagic acid and kaolin is very well known however, this phenomenon is reverted adding α-β2GP1 in a dose-dependent way.[45] Exploring the presence of anti-tPA antibodies in 91 APS patients (68 primary APS and 23 LES-associated SAPS patients) and 914 controls, it was observed that the anti-tPA antibody levels were higher in APS patients. In addition, the activity and concentration of tPA were assayed in 53 of 91 patients; APS patients had higher tPA concentration but lower activity when compared with controls.[107] Finally, in APS patients with or without anti-FXII antibodies, it was found that 50% of these antibodies were able to reduce both, the antigenic levels and the activity of FXII[108] .The meaning of this finding that another mechanism predisposing APS patients to thrombosis is the lack of fibrinolysis activation mediated by FXII.

On the other hand, the presence of anti-activated factor XI antibodies was assayed in 38 APS patients and 30 controls. A higher antibody concentration in APS patients was found and these antibodies were also capable to bind to factor IX (FIX). Although these antibodies did not affect the FIX activity they did alter the inactivation of activated FIX by antithrombin, resulting in an increased FIX activity and a procoagulant state.[62]

From a distinct perspective, the effects of aPLs on the structure and properties of clots have been analyzed. Clots from APS patients are less permeable, denser, with thinner fibers, and more branched than clots from controls and patients with

thrombosis but without APS. Moreover, the clotting process is delayed and the clots are denser and more difficult to lysate in APS patients[109] .An explanation for the higher density of the clots from APS patients could be a higher activity of factor XIII (FXIII), a transglutaminase of endothelial cell origin that cross-links fibrin-fibrin and fibrin-fibronectin fibers giving stability to the clot. In APS patients, increased activity of FXIII in comparison with aPL carriers, controls, and cardiac mechanical valve controls has been described. It is likely that, in APS patients, this high FXIII activity promotes a higher cross-linking of fibrin monomers making the cloth less sensitive to plasmin.[110]

The effects of aPL on plasma factors can be assessed through the blood coagulation tests. APS patients have elevated levels of cell-free DNA and NETs which correlate with the α-β2GP1 titers and that these titers are more increased in LA positive patients and even more in triple-positive patients (aCL+, α-β2GPI+, LA+)[111] .The increased NETs levels are promoted by α-β2GP1 from APS patients by a mechanism involving TLR4[111] . The effect of aPL antibodies on blood coagulation tests is likely related to cell-free DNA and NETs levels. In fact, *in vitro* assays showed that histones are able to prolong the blood coagulation tests (prothrombin, activated partial thromboplastin, and thrombin times as well as diluted Rusel̇ls viper venom test), likely due to their affinity to anionic phospholipids such as phosphatidylserine, and/or their binding to thrombin, and/or to direct inhibition of a contact phase activator. However, by using thrombinography, it has been shown that histones increase the thrombin generation rate and the endogenous thrombin potential without affecting fibrin formation[111,112] .Moreover, a reduced generation of activated PC mediated by histones likely due to the binding to its gamma-carboxyglutamic acid-rich domain of PC has been demonstrated.[112]Moreover, the binding of H4 histone to prothrombin improves their affinity to FXa in absence of phospholipids however, this reaction is inhibited in presence of the other elements of prothrombinase complex (FVa and phospholipids).[113]

A synthesis of the pathophysiological mechanisms described in APS patients are depicted in Figures 3 and 4. Figure 3 shows the effects of histones on platelets, endothelial cells, eosinophils, and soluble factors while Figure 4 depicts a summary of the mechanisms involved in the development of the clinical manifestations of APS.

5. Future Perspectives

The complement system has a pivotal role in the pathophysiology of APS as demonstrated in a mouse model of surgical induced thrombosis where administration of aPLs did not induce thrombosis in C3 and C5 deficient mice or anti-C5 treated normal mice[114] .For years, APS has been treated with a combination of anticoagulants (the milestone of the treatment), steroids, and other drugs such as statins, and HQ. However, up to date, the complement system has not been widely used. The use of new therapeutic monoclonal antibodies

such as anti-C5 and anti-C5aR antibodies may be another complementary therapy to suppress the pathological effects of the activation of the complement. For example, the use of inhibitors of the complement such as eculizumab (a humanized monoclonal antibody that blocks the activation of C5 and further production of the terminal complex C5b-9) in the treatment of catastrophic APS has been reported since 2012. In these reports, catastrophic APS patients with thrombocytopenia, anemia, and elevated D-dimers not responding to immunosuppressive, antiplatelet, and anticoagulant therapy, were treated with eculizumab. After the treatment, anemia and thrombocytopenia resolved and D-dimers levels return to normality.[115,116] Furthermore, the levels of complement activation products like sC5b-9, C4bc, and C3bBbP were decreased in parallel with increased levels of C3 and C4 after treatment with eculizumab.[117] It is likely that inhibitors of complement activation may add to the treatment based on anticoagulant and immunosuppressive agents in an active or severely affected APS patient which does not improve with the first line therapy. Other strategies that may avoid the harmful effects of extracellular traps would be the inhibition of its production by peptidylarginine deiminase 4 (PAD4) inhibitors which consequently will hinder histone signaling through TLR4 and the activation of the placenta, eosinophils, platelets, monocytes, and neutrophils. Of course, these probable positive effects must be demonstrated by culturing eosinophils and neutrophils in the presence of aPLs with or without PAD4 inhibitors. Inhibition of the NO endothelial cell production secondary to over-stimulation through ROS and ET-1 could be targeted using antioxidants. Currently, the role of eosinophils has not been proven although their effects on clot composition have been demonstrated. As occurs with neutrophils, establishing the likely role of eosinophil extracellular traps on the activation of the complement system and the placenta would is warrantied. Whether the role of the eosinophils in the pathophysiology of APS is demonstrated, the therapeutic use of the monoclonal antibody mepolizumab as an additive therapy should be analyzed.

Another possible therapeutic strategy is the use of modified cells targeting aPL-producing cells. For example, T cells with chimeric receptors for antigens (CAR-T cells) may recognize the complementary determining regions in the aPLs-producer cells (plasmablasts or memory B cells) suppressing the production of aPLs. Also, in the same line, using immunotoxins cocktails could be explored in these patients.

Intravenous immunoglobulin (IVIg) may be another therapeutic candidate for the treatment of APS at least in the acute, thrombotic phase of the disease. IVIG, a pooled IgG purified from plasma from healthy donors, could be helpful because: IgG has a concentration-dependent catabolism and, as a consequence, the more is infused to the patient the more IgG is catabolized however, the optimal dose to achieve a significant reduction of aPLs must be carefully assessed; hypersialylation on IgG converts this antibodies to an anti-inflammatory factor because its decreased affinity for the activation of $Fc_{\gamma}Rs$ and its increased affinity to the inhibitory $Fc_{\gamma}R$ ($Fc_{\gamma}RIIB$); finally, the sialylated fraction of IgG in healthy plasma is between

4-14% of the total IgG, the infusion of IVIg in APS patients could be potentially beneficial.

6. Conclusion

APS is a hematological autoimmune disease characterized by thrombotic and obstetric severe manifestations. aPLs are the etiopathogenic agents responsible for the thrombotic and obstetric events complicating and defining the APS. These autoantibodies induce a procoagulant, pro-inflammatory, pro-adhesive phenotype in the endothelium and the release of NETs, sensitize platelets to a second stimulus, inhibit the fibrinolysis, and increase the activity of procoagulant proteins inducing a prothrombotic status. Moreover, aPLs generate oxidative stress, lipoperoxidation, and placenta inflammation. On the other hand, activation of the complement system and the induction of extracellular traps both play a fundamental role in APS, nevertheless, other mechanisms as the decreased bioavailability of NO, hyper-generation of ROS and ET-1, altered patterns of VWF multimers and their longer half-life as well as increased plasma levels of FXIII, are all factors that trigger the characteristic obstetric and thrombotic events of APS.

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