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# Antiphospholipid Antibodies Syndrome and Reproductive Failures: New Therapeutic Trends Beyond Aspirin and Heparin

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Additional information is available at the end of the chapter

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## 1. Introduction

The most investigated thrombophilia related to obstetrical complications is the antiphospholipid antibodies syndrome (APS), also known as Hughes' syndrome. APS is characterized by recurrent thrombosis (arterial or venous, or both) and/or morbidity during pregnancy (losses during early and late pregnancy and pre-eclampsia) associated with moderate to high plasma levels of antiphospholipid (aPL) antibodies (anticardiolipin antibodies, antibodies to  $\beta 2$  glycoprotein I [ $\beta 2$ GPI] or lupus anticoagulants) [1-2].

According to the last International consensus statement for APS diagnostic criteria, in order to make diagnosis of the syndrome, the combination of at least one clinical and one laboratory criterion is required [2] (Table 1).

Since aPL antibodies have thrombogenic properties, intraplacental thrombosis with maternal-fetal blood exchange impairment was traditionally suggested to be the main pathogenic mechanism responsible of fetal loss in patients with APS, providing the rationale for the use of aspirin or heparin to prevent adverse pregnancy outcomes in APS [3-5].

Although the management of aPL antibodies-positive pregnant patients is controversial due to the limited well-designed controlled trials, the current recommendation is to use low-dose aspirin and prophylactic or therapeutic doses of heparin for patients fulfilling the updated Sapporo APS classification criteria [2] and no treatment for asymptomatic (no history of pregnancy complications and/or thrombosis) persistently aPL antibodies-positive patients [6].

Clinical criteria	Laboratory criteria
<p>Vascular thrombosis</p> <ul style="list-style-type: none"><li>• One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ.</li><li>• Thrombosis should be supported by objective validated criteria—ie, unequivocal findings of appropriate imaging studies or histopathology. For histopathological support, thrombosis should be present without substantial evidence of inflammation in the vessel wall.</li></ul> <p>Pregnancy morbidity, defined by one of the following criteria:</p> <ul style="list-style-type: none"><li>• One or more unexplained deaths of a morphologically healthy foetus at or beyond the 10th week of gestation, with healthy foetal morphology documented by ultrasound or by direct examination of the fetus.</li><li>• One or more premature births of a morphologically healthy newborn baby before the 34th week of gestation because of: eclampsia or severe preeclampsia defined according to standard definitions or recognized features of placental failure.</li><li>• Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomical or hormonal abnormalities and paternal and maternal chromosomal causes excluded. In studies of populations of patients who have more than one type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of patients according to one of the three criteria.</li></ul>	<ul style="list-style-type: none"><li>• Lupus anticoagulant 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 (Scientific Subcommittee on lupus anticoagulant/ phospholipid-dependent antibodies).</li><li>• Anticardiolipin antibody of IgG or IgM isotype, or both, in serum or plasma, present in medium or high titres (ie, <math>\geq 40</math> GPL or MPL, or greater than the 99th percentile) on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.</li><li>• Anti-<math>\beta 2</math>-glycoprotein 1 antibody of IgG or IgM isotype, or both, in serum or plasma (in titres greater than the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures.</li></ul>

**Table 1.** Revised diagnostic criteria of APS [2].

In the last years, progress has been made in characterizing the molecular basis of aPL antibodies pathogenicity, which includes direct effects on platelets, endothelial cells and monocytes as well as activation of complement. Furthermore, it has been widely shown that pregnancy loss cannot be attributed exclusively to placental thrombosis and that other pathogenic mechanisms like functional trophoblast impairment, angiogenesis inhibition or complement-mediated placental injury may occur (see more below). Based on these findings, novel therapeutic targets are currently being explored for APS in order to address the unmet needs of better, safer and ideally targeted therapy.

This chapter points to resume the known mechanisms of aPL antibodies-mediated pregnancy impairment, the proven therapies and the new therapeutic perspectives to ameliorate obstetric outcomes of pregnant women with APS.

**2. Adverse pregnancy outcomes associated to APS**

Beyond thromboses, obstetric complications are the other main features of APS. Such association is supported by several epidemiological studies and experimental models showing that passive transfer of aPL IgG induces fetal losses and growth retardation in pregnant naive mice, giving the proof that aPL antibodies are involved in determining the clinical manifestations of the syndrome [7-9]. The most common adverse pregnancy outcome associated to APS is

recurrent miscarriage, defined as the occurrence of three or more unexplained consecutive miscarriages before the 10th week of gestation. Other obstetric features of APS are unexplained fetal deaths, occurring at or beyond the 10th week of gestation, and premature births of a morphologically healthy newborn baby before the 34th week of gestation because of eclampsia or severe preeclampsia [10].

Recurrent miscarriage occurs in about 1% of the general population attempting to have children [11] and about 10–15% of women with recurrent miscarriage are diagnosed with APS [12,13]. Fetal death in the second or third trimesters of pregnancy occurs in up to 5% of unselected pregnancies [14] but it is less likely as pregnancy advances [15]. Although fetal death occurs significantly most often in APS [16], the overall contribution of this syndrome to its pathogenesis is unknown, because of the effect of other possible contributing factors such as underlying hypertension or pre-existing comorbidities, like systemic lupus erythematosus (SLE) or renal diseases. Furthermore, it has been observed that pregnant women with diagnosis of APS are at increased risk for developing preeclampsia or placental insufficiency.

Even if it is still unknown the precise relationship between aPL antibodies and the occurrence of obstetric complications, aPL antibodies seem to be detectable in 11–29% of women with preeclampsia, compared with 7% or less in controls and in 25% of women delivering growth restricted fetuses [17]. Furthermore, results from prospective cohort studies indicate that among pregnant women with high concentrations of aPL antibodies, 10–50% develop preeclampsia, and more than 10% of these women deliver infants who are small for gestational age [17].

A significant correlation between aPL positivity and increased risk of fetal loss has also been established. In particular, the strongest association has been observed with ACA positivity, followed by annexin V, lupus anticoagulant and anti- $\beta$ 2GPI. In addition, lupus anticoagulant seems to be significantly associated with early pregnancy loss compared to late pregnancy loss [18].

### **3. Pathogenetic mechanisms mediated by aPL antibodies and related therapeutic approaches**

#### **3.1. Vascular and placental thrombosis**

The molecular mechanisms underlying thrombosis and fetal death in APS have long been investigated. The main target antigens reported in patients with APS include  $\beta$ 2GPI/cardiolipin, prothrombin and annexin V [19]. Other putative antigens are thrombin, protein C, protein S, thrombomodulin, tissue plasminogen activator, kininogens (high or low molecular), prekallikrein, factor VII/VIIa, factor XI, factor XII, complement component C4, heparan sulfate proteoglycan, heparin, oxidised low-density lipoproteins [19,20]. The main autoantigens are attracted to negatively charged phospholipids exposed on the outer side of cell membranes in great amounts only under special circumstances such as damage or apoptosis (e.g. endothelial cell) or after activation (e.g. platelets) [19].

Endothelial cells, activated by aPL antibodies with anti- $\beta$ 2GPI activity, express adhesion molecules such as intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, E-selectin, and both endothelial cells and monocytes upregulate the production of tissue factor (TF) [21]. All at once, activated platelets increase expression of glycoprotein IIb-IIIa and synthesis of thromboxane A<sub>2</sub>, determining a procoagulant state [21-25].

Additional mechanisms promoting clot formation could be represented by interaction of aPL antibodies with proteins implicated in clotting regulation, such as annexin A5, prothrombin, factor X, protein C and plasmin [20,21,26].

Recent results from studies in mice highlight the role of inflammation in the pathogenesis of APS, showing a central role for complement activation in determining thrombosis and fetal loss induced by aPL antibodies [27,28]. Because many individuals with high aPL antibodies titers remain asymptomatic, a "second hit" hypothesis has been proposed. It is likely that in the aPL antibodies-induced vascular procoagulant state, activation of the complement cascade might close the loop and provoke thrombosis, often in the presence of a second hit, like tobacco, inflammation, or oestrogens [26,29,30].

Starting from the observation of the intravascular aPL antibodies-mediate clot formation, initially, intraplacental thrombosis was considered the main pathogenic mechanism mediating fetal loss in APS. This hypothesis of placental damage was supported by the finding of thrombosis and infarction in placentas from women with APS and by the demonstration of aPL antibodies capability to induce a procoagulant state in vitro through several mechanisms, including their ability (specifically, anti- $\beta$ 2GPI antibodies) to disrupt the anticoagulant annexin A5 shield on trophoblast and endothelial cell monolayers [20,31,32]. Supporting the in vitro findings, a significantly lower distribution of annexin A5 covering the intervillous surfaces was found in the placentas of aPL antibodies-positive women in comparison with normal controls [33]. These observations supported the introduction of heparin in the prevention of fetal loss in APS, preferred to oral anticoagulant therapies for its safer profile for the fetus. Indeed, it was demonstrated that heparin, at concentrations that are reached therapeutically in vivo, greatly enhanced the plasmin-mediated cleavage of  $\beta$ 2-GPI. Considering that the cleaved forms of  $\beta$ 2-GPI cannot bind to PL and may be cleared more rapidly from the circulation than native  $\beta$ 2-GPI [34], interaction with heparin should greatly reduce the prothrombotic effects of anti- $\beta$ 2-GPI antibodies. Yet, some clinical trials confirmed heparin therapy effectiveness in improving pregnancy outcomes in APS patients [35].

Nevertheless, since recurrence of thrombotic events occurs despite the therapy and thrombosis cannot account for all of the histopathologic findings in placentae from women with APS, other mechanisms of reproductive impairment were supposed to be involved [36,37].

### **3.2. Defective placentation**

#### *3.2.1. Trophoblast invasiveness impairment*

New aPL antibodies-mediated pathogenic mechanisms have been proposed during the last fifteen years: anti- $\beta$ 2GPI antibodies seem to bind directly the maternal decidua and the invading trophoblast, determining defective placentation.



On the fetal side,  $\beta$ 2GPI has been shown to be expressed on trophoblast cell membranes, explaining the placental tropism of anti- $\beta$ 2GPI antibodies. Being a cationic plasma protein,  $\beta$ 2GPI has been suggested to bind to exposed phosphatidylserine on the external cell membranes of trophoblasts undergoing syncytium formation [38].

$\beta$ 2GPI-dependent antibodies can adhere to human trophoblast cells in vitro [39], consistently with the hypothesis that the visibility of anionic PLs on the external cell surface during intertrophoblastic fusion might offer a useful substrate for the cation PL-binding site [40,41]. The binding to anionic structures induces the expression of new cryptic epitopes and/or increases the antigenic density, two events that are apparently pivotal for the antibody binding [42]. In vitro studies with both murine and human monoclonal antibodies, as well as with polyclonal IgG antibodies from APS patients, have clearly demonstrated a binding to trophoblast monolayers [39,43]. Interestingly, antibodies obtained from patients with APS, once bound, can affect the trophoblast functions in vitro, inducing cell injury and apoptosis, inhibition of proliferation and formation of syncytia, decreased production of human chorionic gonadotrophin, defective secretion of growth factors and impaired invasiveness [39].  $\beta$ 2GPI-dependent aPL antibodies seem, therefore, to represent the main pathogenic autoantibodies in obstetrical APS.

The most important mechanism by which heparin acts protecting placenta in APS seems to be its ability to prevent the binding of aPL antibodies to trophoblast cells. Indeed, using an expression/site-directed mutagenesis approach, Guerin demonstrated that the primary heparin-binding site of  $\beta$ 2-GPI is the positively charged site located within the fifth domain of the protein, which also binds to PL [44]. Furthermore, we demonstrated that heparin reduces the aPL antibody binding to trophoblast cells in vitro and that it is able to restore placental invasiveness and differentiation [45,46].

Recent findings have underlined a further mechanism by which aPL antibodies binding to human trophoblast could affect its functions: the aPL antibodies-mediated reduction of placental Heparin-Binding Epidermal Growth Factor-like growth factor (HB-EGF) expression. HB-EGF is a member of the EGF family [47,48]. It has been shown to induce an invasive trophoblast phenotype in human and mouse blastocysts [49,50] and to initiate the molecular and cellular changes characteristic of decidualization in mice [51]. HB-EGF is expressed in the human placenta during the first trimester, primarily within the villous trophoblast, but also in the extravillous cytotrophoblast, predominantly at the sites of cytotrophoblast extravillous invasion [52]. Women with preeclampsia and infants small for gestational age display decreased placental expression of HB-EGF [53], strongly suggesting an association between HB-EGF down-regulation, poor trophoblast invasion, and failed physiologic transformation of the spiral arteries occurring in these disorders.

Interestingly, also in placental tissues obtained from women with APS, we found reduced expression of HB-EGF [54]. Furthermore, we showed that polyclonal and monoclonal aPL antibodies bind trophoblast monolayers in vitro significantly reducing the synthesis and the secretion of HB-EGF [55]. The ability of exogenous recombinant HB-EGF to reduce the aPL antibodies mediated effects on trophoblast cells supports the hypothesis of a key pathogenic role of this molecule in mediating APS-related adverse pregnancy outcomes.

We also observed that the addition of heparin inhibited aPL antibodies binding and restored HB-EGF expression in a dose-dependent manner [54]. These findings suggest that the reduction of aPL antibodies-mediated HB-EGF represents an additional mechanism that is responsible for the defective placentation associated with APS and provide one more proof that heparin works in protecting pregnancy from aPL antibodies-induced damage by inhibiting antibody binding to trophoblast cells.

### 3.2.2. Endometrial angiogenesis inhibition

On the maternal side, endometrial endothelial angiogenesis inhibition has been suggested to be a further aPL antibodies-mediated mechanism of placental damage. Indeed, aPL antibodies have been observed to selectively bind in vitro to endothelial cells isolated from human endometrium (HEEC) and to inhibit endothelial cell differentiation into capillary-like tubular structures, by reducing matrix metalloproteinase-2 (MMP-2) activity and vascular endothelial growth factor (VEGF) secretion, via a suppression of intracellular NF $\kappa$ B DNA binding activity [55]. Such an aPL antibodies-mediated inhibition of angiogenesis has also been confirmed in vivo in a murine model, showing a reduced angiogenesis in subcutaneous implanted angioreactors in aPL antibodies-inoculated mice [55]. Since it is well known that endometrial angiogenesis and decidualization are fundamental prerequisites for successful implantation and placental development, aPL antibodies-inhibition of this central process provides an important additional mechanism able to explain the association between APS and pregnancy complications associated to placental failure, like miscarriage, fetal growth restriction and preeclampsia.

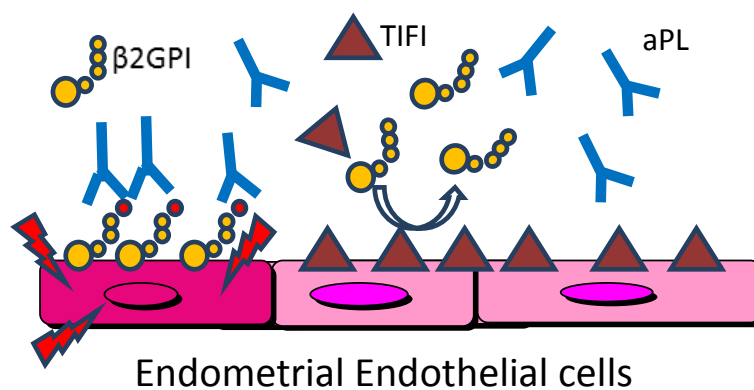
Recently, we investigated whether two low molecular weight heparins (LMWHs), tinzaparin and enoxaparin, have an effect on the aPL antibodies-inhibited endometrial angiogenesis. We demonstrated that the addition of the two LMWHs prevents aPL antibodies-mediated inhibition of HEEC angiogenesis, both in vitro and in vivo in a murine model, and that LMWHs are able to restore Nuclear Factor- $\kappa$ B (NF- $\kappa$ B) and/or STAT-3 activity, VEGF secretion and MMP-2 activity inhibited by aPL antibodies [56]. A noteworthy aspect of our results was that tinzaparin improved aPL-inhibited in vitro angiogenesis and STAT-3 activity more effectively than enoxaparin but it is difficult to explain this difference between the two LMWHs and caution is necessary in extrapolating the obtained results.

In conclusion, this study provides the demonstration of a beneficial effect of LMWHs on the aPL antibodies-inhibited HEEC angiogenesis offering a new mechanism whereby treatment with heparin protects early pregnancy in APS.

Beyond heparin administration, recently, a new therapeutic perspective has been investigated to provide a safer profile therapy to prevent aPL antibodies-mediated angiogenesis inhibition. During the last decade, several groups attempted to neutralize the pathogenic effect of aPL antibodies by using synthetic peptides reproducing the  $\beta$ 2GPI epitopes recognized by these antibodies [57,58].

An alternative approach by other groups employed synthetic portions of the whole molecule able to compete with  $\beta$ 2GPI in its binding to the natural cell targets, ultimately inhibiting its

expression and the recognition by specific autoantibodies [59,60]. It has been shown that a twenty amino acid synthetic peptide of viral origin spanning Thr101-Thr120 of ULB0-HCMVA from human Cytomegalovirus (TIFI) shares similarity with a 15 amino acid sequence rich in lysines in the Vth domain of  $\beta$ 2GPI located in the PL-binding site [61]. TIFI inhibits the binding of FITC-conjugated  $\beta$ 2GPI to human endothelial cells and murine monocytes in vitro. It has been demonstrated that this peptide is not recognized by aPL antibodies but it is able to reverse the aPL antibodies-mediated thrombosis in mice [59]. Furthermore, TIFI was recently shown to inhibit also the binding of monoclonal human  $\beta$ 2GPI-dependent aPL antibodies to human trophoblast in vitro, and, more importantly, it has been observed that repeated injections of TIFI protected pregnant naïve mice from fetal loss and growth retardation induced by passive infusion of human aPL IgG [62-64]. Since  $\beta$ 2GPI was shown to bind endothelial cells and trophoblasts through the PL-binding site, it has been suggested that TIFI might compete with  $\beta$ 2GPI in the binding to cell membranes so reducing the amount of the target antigen available for aPL antibodies and ultimately might reverse the autoantibody-mediated pro-thrombotic or fetal loss effects [59,61-65] (Figure 1).



**Figure 1.** TIFI competes with  $\beta$ 2GPI in the binding to HEEC membranes reducing the amount of the target antigen available for aPL antibodies and ultimately reverses the autoantibody-mediated fetal loss [66].

Based on these recent discoveries, we examined the ability of TIFI to interfere with aPL antibodies-mediated inhibition of human endometrial angiogenesis and we observed that the peptide is able to revert the anti-angiogenic effects mediated by a  $\beta$ 2GPI-dependent aPL monoclonal IgG on HEEC both in vitro and in vivo [66]. We showed for the first time that TIFI is able to prevent the effects of  $\beta$ 2GPI-dependent aPL antibodies on human endometrial endothelial cell (HEEC) functions. In fact, the addition of TIFI to HEEC cultures restores VEGF expression and MMP-2 gelatinolytic capacity affected by  $\beta$ 2GPI-dependent monoclonal aPL antibodies [66]. This finding is also confirmed by parallel experiments performed in a murine model of in vivo angiogenesis [66].

### 3.3. Inflammation

A physiological pregnancy development requires a fine regulation of the maternal immune response during embryo implantation. Acute inflammatory events are recognized causes of



adverse pregnancy outcomes, and proinflammatory mediators, such as complement, tumor necrosis factor (TNF), and CC chemokines, have been shown to play a role in animal models of aPL antibodies-induced fetal loss [67].

Recently, the excessive activation of complement system at the fetal-maternal interface has been proposed as an additional aPL antibodies-mediated mechanism of placental damage responsible for negative outcome in APS. In pregnant murine model of APS, Girardi et al. demonstrated that aPL antibodies, preferentially targeted at deciduas and placentas, activate the complement system through the classical pathway, leading to generation of potent anaphylatoxins and mediators of effector-cell activation. The recruitment of inflammatory cells accelerates local alternative pathway activation and creates a proinflammatory amplification loop that enhances complement component 3 (C3) activation and deposition, generates additional C3a and C5a and results in further influx of inflammatory cells into the placenta [8].

The pathogenic mechanism of complement-mediated fetal loss induced by aPL antibodies is also supported by the protection that deficiency in complement components confers on the animals, or that follows from in vivo inhibition of complement [68,69]. Hence, the complement system could be a potential therapeutic target in aPL-positive patients.

Interestingly, Girardi and co-workers demonstrated that treatment with heparin prevent complement activation in vivo and protect mice from pregnancy complications induced by aPL antibodies [28]. Such low doses of heparin, lacking anticoagulant effects, inhibited inflammatory responses at the level of leukocyte adhesion and influx and limited tissue injury [70-73]. Neither fondaparinux nor hirudin, other anticoagulants without known effects on complement [74], prevented pregnancy loss, demonstrating that anticoagulant therapy gives insufficient protection against APS-associated miscarriage [28].

Moreover, it has been demonstrated that heparin possesses the ability to inhibit lipopolysaccharide-induced proinflammatory cytokines (tumour necrosis factor  $\alpha$ , interleukin 6, 8 and 1 $\beta$ ) [75], involved in recurrent fetal loss of a murine model of APS [76].

Administration of intravenous immunoglobulin (IVIG) has been proposed as a possible treatment to prevent pregnancy loss in APS. IVIG may act by modulating the effect of cytokines as well as by inhibiting the action of pathological antibodies by either the interaction of the Fc portion of immunoglobulin with Fc receptors or the Fab receptors, or by passively acting as anti-idiotypic. IVIG also modulates the activation and effector functions of B and T lymphocytes, neutralizes pathogenic autoantibodies, and interferes with antigen presentation. Furthermore, the anti-inflammatory effect of IVIG may be due to interaction with the complement system [77].

IVIG have been shown to reduce the number of fetal resorptions in mice in which APS had been induced by immunization with aPL [78]. However, in a multicenter placebo controlled pilot study, the administration to pregnant women with APS of IVIG associated to low-dose aspirin plus heparin did not improve obstetric or neonatal outcomes beyond those achieved with a heparin and low-dose aspirin regimen [79].

Recently, Blank and coworkers demonstrated that anti-anti-  $\beta$ 2GPI specific IVIG (sIVIG) are able to improve significantly the pregnancy outcome in BALB/c mice passively infused with

anti- $\beta$ 2GPI antibodies. They also observed that incubation of sIVIG restored the anti- $\beta$ 2GPI antibodies-inhibited invasiveness of both JAR cells and human trophoblast cells in vitro. Based on these results, APS sIVIG may be considered as potential specific therapeutic safe compound for developing a treatment for APS patient's early fetal loss [80].

Much research is still needed on the effects and proper indications for IVIG in reproductive failure. However, its prohibitive cost will probably prevent it ever becoming a first-line drug and its place is probably best reserved for severely affected patients who cannot be helped by simpler modes of treatment.

A new potential mechanism of aPL antibodies-mediated fetal loss linking TF and complement activation has been recently proposed. TF, best known as the primary cellular initiator of blood coagulation, also contributes to a variety of biological processes. In particular, TF is involved in inflammation and cell injury processes by interacting with protease-activated receptors (PARs) [81], a subfamily of related G protein-coupled receptors. Complexes of TF-FVIIa and TF-FVIIa-factor Xa (FXa) as well as FXa and thrombin induce pro-inflammatory signals by activating PARs and inducing the expression of TNF- $\alpha$ , interleukins, and adhesion molecules [82–84].

Increased expression of TF on monocytes from patients with APS has been reported [85–87] and in vitro studies support a direct role for aPL antibodies in inducing monocyte TF expression. Indeed, incubation of normal monocytes with IgG from patients with APS or monoclonal aPL antibodies has been shown to induce TF expression and activity in these cells [88–90]. In particular, anti- $\beta$ 2GPI human monoclonal antibodies derived from APS patients enhance monocyte TF mRNA and activity in a  $\beta$ 2GPI-dependent fashion [91]. Similarly, aPL antibodies (and specifically anti- $\beta$ 2GPI antibodies) have been shown to induce TF, along with inflammatory cytokines and adhesion molecules, on endothelial cells [92–94].

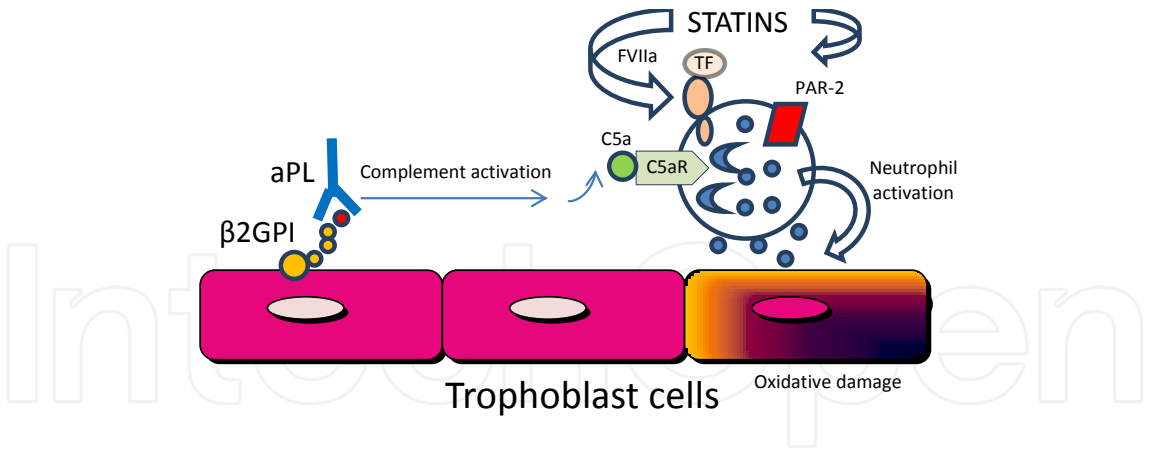
Ritis et al. reported that complement activation is a fundamental process in determining aPL-induced increase of TF expression on neutrophils. Indeed, in this study the authors showed that complement component C5a induced TF synthesis and expression on neutrophils through interaction with C5a receptor [95].

Based on this finding, Redecha et al. investigated whether TF contributes to aPL antibodies-induced fetal loss in mice and observed that mice treated with aPL antibodies showed strong TF staining throughout the decidua and on embryonic debris [96]. Surprisingly, neither increase in fibrin staining nor thrombi was associated with increased TF staining in deciduas from aPL antibodies-treated mice [96]. Moreover, anticoagulation with hirudin and fondaparinux was not sufficient to prevent pregnancy loss in this model. Neither TF increase nor fetal death was observed in mice deficient in complement component C5a receptor (C5aR) treated with aPL antibodies, demonstrating the importance of C5a-C5aR interaction in TF expression and fetal death in this model of APS. To assess the importance of TF in aPL antibodies-induced fetal injury, Redecha et al. inhibited TF with a monoclonal anti-mTF antibody 1H1 [96]. TF blockade prevented fetal death in aPL antibodies-treated mice also diminishing complement component C3 deposition and neutrophil infiltration in deciduas [96].

Furthermore, Redecha et al. investigated the intracellular pathway of TF complement-mediated activation, observing a 5-fold increase in PAR-2 mRNA expression in neutrophils of aPL antibodies-treated mice when compared to untreated mice or mice treated with control antibodies [97] as well as an increased reactive oxygen species (ROS) production and phagocytosis in neutrophils from aPL antibodies-treated mice. Interestingly, neutrophils from PAR-2 deficient (PAR-2<sup>-/-</sup>) mice displayed a dramatic reduction of neutrophil activation in aPL antibodies-treated mice [97]. Also ROS production and phagocytosis were significantly reduced in neutrophils from PAR-2<sup>-/-</sup> mice treated with aPL antibodies compared with aPL antibodies-treated wild-type mice. Specific monoclonal antibody 10H10 that selectively blocks TF-VIIa signaling through PAR-2, but not antibody 5G9 that only prevents TF procoagulant activity, prevented oxidative burst and increased phagocytosis in mice treated with aPL antibodies [97]. These results are in accordance with the previous observation that anticoagulation does not rescue pregnancies in APS [43]. In addition, neutrophils from TF  $\delta$ CT/ $\delta$ CT mice that carry a mutation in the cytoplasmic domain involved in PAR-2 signaling did not show increased oxidative stress and phagocytosis in response to aPL antibodies [97]. This data reinforces the idea that TF-FVIIa signaling is required for neutrophil activation and fetal injury in APS.

The possible application of statins in the prevention of aPL antibodies-related adverse pregnancy outcomes arises from their ability to suppress TF expression in various cell types [98]. Knowing that TF is a crucial mediator in aPL antibodies-induced pregnancy loss and that statins diminish TF expression, Girardi et al. investigated whether statins could prevent pregnancy loss in aPL antibodies-treated mice and demonstrated that simvastatin and pravastatin prevented fetal loss by reducing TF and PAR-2 expression on neutrophils, thus preventing neutrophil activation [99]. aPL antibodies-dependent increase in free radical-mediated lipid peroxidation in decidual tissue was also ameliorated by treatment with statins [99] (Figure 2). These results suggest that statins prevent aPL antibodies-induced neutrophil activation, thus protecting trophoblasts from oxidative damage and rescuing the fetuses. However, given that statins are not major teratogens [100,101] and considering the beneficial effects of statins in animal studies, statins should be considered as a possible treatment for women with aPL antibodies-induced pregnancy complications. Clinical trials are needed to confirm the effectiveness of its application to humans.

Mulla et al. Investigated further mechanisms characterizing the inflammatory process occurring *in vitro* in trophoblast cells after aPL antibodies binding. Indeed, they demonstrated that anti- $\beta$ 2GPI antibodies trigger an inflammatory response in trophoblast, characterized by increased secretion of IL-8, MCP-1, GRO- $\alpha$  and IL-1 $\beta$ , and that this occurs in a TLR-4/MyD88-dependent manner [102]. At high concentrations, these antibodies also induce caspase-mediated cell death. This was attenuated upon disabling of the MyD88 pathway, suggesting that anti- $\beta$ 2GPI-induced inflammatory mediators compromise trophoblast survival by acting in an autocrine/paracrine manner. Enhanced IL-8, GRO- $\alpha$  and IL-1 $\beta$  secretion also occurred when trophoblast were incubated with antibodies from patients with APS. Heparin attenuated the anti- $\beta$ 2GPI antibody-mediated cell death, and also the pro-inflammatory response, but only at high concentrations [102]. These findings demonstrate that aPL antibodies triggers a



**Figure 2.** Statins prevent C5a-induced upregulation of TF and PAR2 expression, thereby inhibiting the release of reactive oxygen species and suppressing amplification of complement activation by factors released from neutrophils. Modified from Girardi G. et al., *Journal of Reproductive Immunology* 2009 [99].

placental inflammatory response via the TLR-4/MyD88 pathway, which in turn compromises trophoblast survival. Thus, the TLR-4/MyD88 pathway may provide a new therapeutic target to improve pregnancy outcome in APS.

4. Conclusions

Heparin, associated to low dose aspirin, is considered nowadays the gold standard for the prevention of obstetric complications in pregnant women with APS. Yet, heparin therapy administered at subanticoagulant doses has improved pregnancy outcomes in some trials of APS patients [35]. Several studies have proposed different mechanisms of action able to explain the beneficial effect of heparin in preventing adverse pregnancy outcomes associated to APS (Table 2).

- ↓ aPL antibodies binding to trophoblast cells
- ↑ Cleavage of β2GPI
- ↓ Complement activation
- ↓ Trophoblast cells apoptosis
- ↑ Trophoblast cells invasiveness and differentiation
- ↑ HB-EGF expression
- ↑ Endometrial angiogenesis

**Table 2.** Heparin mediated mechanisms of placental protection from aPL antibodies.

However, in women with APS and history of obstetric failures, pregnancy complications recur in the 15% of cases despite the therapy.

In the last years new mechanisms of aPL-mediated pregnancy impairment, like inflammation and angiogenesis inhibition, and more specific target therapies have being examined in order

to treat more selectively the aPL antibodies-induced placental damage and to ameliorate the reproductive performance of women with APS. Studies on humans are required to verify the safety profile and effectiveness of new therapies proposed.

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