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Pathogenesis of the obstetric antiphospholipid syndrome: the key role of beta 2 glycoprotein I

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Abstract

Antiphospholipid syndrome (APS) is defined by recurrent pregnancy morbidity and/or vascular thrombosis associated with the persistent presence of antibodies against anionic phospholipid-binding proteins. Beta 2 glycoprotein I (β2GPI) and prothrombin (PT) are the major antigens for antiphospholipid antibodies (aPL) detectable by functional coagulation [lupus anticoagulant (LA)] or solid-phase assays [anti-β2GPI-dependent cardiolipin (aCL) and anti-β2GPI]. β2GPI-dependent aPL are responsible for the positivity of the three classification laboratory criteria. While medium/high titers of antibodies against β2GPI are risk factors for both the vascular and the obstetric manifestations of APS, persistent low titers are also associated with pregnancy complications. There is evidence from animal models of aPL-dependent fetal loss and from *in vitro* systems that β2GPI-dependent aPL can be pathogenic. β2GPI is physiologically found in large quantities at the placental level being available for the specific antibodies circulating in the maternal blood. Once bound to the protein, the antibodies trigger a local inflammation via the activation of the complement cascade and affect trophoblast and decidual function. The final result is represented by defective placentation, while thrombotic events are apparently less important. β2GPI is a pleiotropic molecule with scavenging properties towards several molecules including apoptotic material and displays anti-oxidant activity. These functions may explain the β2GPI placental localization in an area of intensive tissue remodeling and low oxygen tension. Since β2GPI interacts also with the complement and the coagulation cascade, its binding with specific antibodies may affect the physiology of placentation in several ways.

Keywords

Antiphospholipid syndrome, beta 2 glycoprotein I, miscarriages, placenta

Introduction

Obstetric antiphospholipid syndrome

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized either by recurrent pregnancy morbidity or vascular thrombosis and by the persistent presence of antibodies against negatively charged phospholipid (PL)-binding proteins [1].

The APS can manifest as primary APS or associated with another autoimmune disease, most frequently systemic lupus erythematosus. Usually, APS displays both vascular and obstetric events, but pure vascular or obstetric variants have also been described [2].

The classification of APS can be formally made when the patient fulfills at least one clinical and one laboratory criterion according to the Sydney-Sapporo criteria. The classification criteria are currently used for the diagnosis of APS as well. The formal classification laboratory tests for the detection of antibodies against anionic PL-binding proteins are the lupus anticoagulant (LA), the beta 2 glycoprotein I (β2GPI)-dependent anti-β2GPI-dependent cardiolipin (aCL), and the anti-β2GPI assays [1].

Pregnancy complications represent one of the formal clinical criteria for classification; in particular, three patterns of pregnancy morbidity are identified:

1. ≥ 3 unexplained consecutive pre-embryonic or embryonic miscarriages before 10 weeks of gestation, with maternal and paternal factors (such as anatomical, hormonal or chromosomal abnormalities) excluded;

2. ≥ 1 unexplained death of a morphologically normal fetus beyond 10 weeks of gestation;

3. ≥ 1 premature delivery of a morphologically normal fetus < 34 weeks of gestation because of severe preeclampsia, eclampsia, or recognized features of placental insufficiency [1, 3, 4].

The so "called" antiphospholipid antibodies

There is a general agreement that antiphospholipid antibodies (aPL) do not bind directly to anionic PLs but a complex between PL and PL-binding proteins. β2GPI was identified as the most relevant co-factor for aPL [5, 6]. β2GPI-dependent antibodies bind the target antigen: i) when complexed with cardiolipin (CL)-coated plates (i.e. aCL assay) or ii) when the plates are directly coated with β2GPI. In both conditions, β2GPI displays conformational changes and/or increases its antigenic density, offering the correct antigen structure to the antibodies [5, 6]. Moreover, there is evidence that both anti-β2GPI and anti-prothrombin (PT) antibodies are responsible for the largest part of positivity for the LA [5, 6]. Altogether these data strongly support the fact that β2GPI-dependent aPL can be responsible for the positivity in all the three formal laboratory classification (and diagnostic) tests for APS.

The same β2GPI-dependent aPL are thought to be pathogenic mediating both the vascular thrombosis and the placental damage associated with the obstetric APS (see section "The pathogenic role of β2GPI-dependent antibodies in the obstetric APS").

The physiological role of β2GPI

As stated above, β2GPI [or apolipoprotein H (APOH)] is widely accepted as the main antigen for aPL, but its physiological role is still a matter of research. β2GPI is a pleiotropic protein involved in several biological pathways, including inflammation and coagulation [7]. It is present in large amounts in the plasma (0.2 mg/mL) and is highly conserved across the animal kingdom suggesting that it may have crucial biological functions. The protein is produced in the liver and displays five domains known as short consensus repeats or complement control protein repeats [8].

There is evidence that β2GPI exerts a scavenging role on several molecules. By binding lipopolysaccharide (LPS), it exerts a protective effect in a murine model of LPS-mediated inflammation [9], and interacts with viruses and bacteria [10]. β2GPI binds apoptotic material and favors the uptake of apoptotic cells by professional phagocytes [11]. β2GPI forms complexes with oxidized low-density lipoprotein (OxLDL), at least in part because of its cationic charge, and favors their clearance mitigating the OxLDL toxicity [12].

Initially, β2GPI was reported as a natural anticoagulant acting as a plasma inhibitor of the intrinsic coagulation pathway and of the platelet activation [13]. The production of anti-β2GPI autoantibodies was thought to switch the hemostatic balance towards a pro-coagulant state. However, APOH null mice have a net anticoagulant phenotype compared to wild-type mice, unexpectedly suggesting a procoagulant property of β2GPI *in vivo* [14]. Additional studies showed that the molecule may exert both anticoagulant and procoagulant effects, making it even more complex to draw definitive conclusions on its true impact on the coagulation [7].

There is evidence that β2GPI plays also a role as a complement regulator [15]. Through its effect on the complement and on the coagulation cascades, β2GPI is involved in several biological pathways of innate and adaptive immunity [12, 16].

Interestingly, β2GPI is directly involved in the oxidative stress system. The redox switch at the level of the domain V of the molecule is crucial for the anti-oxidant activity [17]. Conditions characterized by important oxidative stress such as ischemia/reperfusion injury or hypoxic state may affect β2GPI levels as shown in animal models of stroke [18].

Proteomic studies reported different β2GPI expression also in related conditions such as in serum samples of coronavirus disease 2019 (COVID-19) patients [19], in platelets from stroke patients [20], and in plasma samples of women with pregnancy complications [21].

The involvement of β2GPI in inflammation, coagulation, and oxidative stress regulation suggests a role of the molecule in the implantation and normal placentation reinforcing the concept that β2GPI goes beyond the mere role of autoantigen for aPL in the APS [7].

In particular, the reduction of β2GPI plasma levels in women with early-onset preeclampsia and the variations in the placental oxygenation during pregnancy support a key anti-oxidant function in normal placentation [21–23]. Consistent with its role in placentation, β2GPI is present in large amounts on trophoblasts in the normal human placenta [24]. Comparable protein localization was reported on the endothelium and trophoblast at the embryo implantation sites in pregnant naive mice [25]. Although β2GPI null mice are fertile and carry viable fetuses to term, the number of viable implantation is significantly reduced, and the fetuses that reach late gestation have markedly lower weight and fetal/placental weight ratio suggesting defective placentation in these animals [26].

Owing to the link between the defective placentation in preeclampsia and other pregnancy disorders and vascular lesions in the arterial system [27], it is useful to speculate on the role of the autoimmune response against β2GPI in maternal vascular damage. For example, T lymphocytes specific for β2GPI have been reported in atherosclerotic plaques of APS patients with systemic lupus erythematosus and found to be responsible for the local production of inflammatory cytokines (i.e. interleukin-17). This finding supports the role of the autoimmune response against β2GPI as a key mechanism for atherosclerotic plaque instability and the possible consequent vascular events [28].

Despite the limited and somewhat controversial data on β2GPI human deficiency, it is worthy to stress that β2GPI null mice display abnormalities in two conditions closely related to human APS: coagulation and pregnancy. Altogether these data support the role of β2GPI in both the physiological and pathological placentation regardless of being the target antigen for aPL.

The pathogenic role of β2GPI-dependent antibodies in the obstetric APS

The role of coagulation in the pathogenesis of obstetric APS is apparently less important than in vascular APS. Although thrombotic lesions can be detected in APS placentae, the finding is not characteristic since it can also be found in conditions unrelated to aPL.

However, vascular remodeling of uterine vessels and/or decidual hypercoagulability have been described even in the absence of clear thrombotic lesions [29]. Pathogenic mechanisms not involving coagulation have consistently been reported to explain the defective placentation associated with APOH in APS [6, 29].

The biodistribution of β2GPI is similar in human and rodent tissues: the molecule cannot be demonstrated on resting endothelium in the vascular tree but it is physiologically present in the placenta tissues [25]. β2GPI can be found on the vascular endothelium only after an inflammatory stimulus. This finding is consistent with the two-hit hypothesis explaining why persistent aPL can be thrombogenic only in the presence of an inflammatory/infectious stimulus [6]. On the contrary, β2GPI is physiologically present on the decidual endothelium, villous and extravillous trophoblasts, and can be available for maternal aPL. Pregnant mice immunized against β2GPI display a β2GPI biodistribution similar to naive pregnant mice; the main difference is that the immunized animals exhibit a significant increase in fetal loss associated with C3 and C9 deposition at the implantation sites as a consequence of the aPL binding [25]. Once bound to placenta β2GPI, aPL may affect trophoblast proliferation and differentiation and induce apoptosis. The same antibodies interfere with angiogenesis and spiral artery development and induce local inflammatory responses when bound to decidual cells and extravillous trophoblasts β2GPI [6].

aPL activate complement, which in turn may switch on the coagulation cascade, ultimately supporting fibrin deposition and placenta vessel thrombosis. The complement activation is supported by the demonstration of the deposits of complement factors in human placentas from APS women. Moreover, complement deficient mice or mice treated with complement blocking molecules are protected from fetal loss induced by passively infused human immunoglobulin G (IgG) aPL [30, 31]. Complement activation triggers a placental inflammatory response, as shown in pregnant naive mice passively infused with large amounts of human IgG aPL [32].

However, an inflammatory signature in the human placental tissue was not confirmed in other studies [30]. Consistent with this finding, the passive transfer of small amounts of IgG aPL still induced fetal resorption in another model of aPL-mediated fetal loss without inflammation [2, 33].

In summary, while there is no doubt about the role of complement activation in obstetric APS, its exact role in fueling placental inflammation is still a matter of research. It is useful to speculate on the kinetic of these inflammatory pathways throughout pregnancy. For example, a transient inflammatory response only at the beginning of the pregnancy could support the placenta damage without histological inflammatory signs in term placentas.

The effect of aPL on trophoblast growth and differentiation may explain early miscarriages. On the other hand, late obstetrical manifestations (e.g., preeclampsia, intrauterine growth restriction, and intrauterine fetal death) can be also related to the failure of extravillous trophoblasts in remodeling spiral arteries, eventually resulting in hypoxic injury.

The prognostic value of β2GPI-dependent antibodies in the obstetric APS

aPL are a risk factor for the clinical manifestations of APS, including adverse pregnancy outcome (APO) [6]. The 2019 European League Against Rheumatism (EULAR) recommendations for the management of APS pregnant women defined a high-risk profile as the persistent presence of LA or double (any combination of LA, β2GPI-dependent aCL or anti-β2GPI antibodies) or triple (all three subtypes) aPL positivity or the presence of persistently high aPL titers [34].

As stated above, β2GPI-dependent antibodies are responsible for all the three formal classification/diagnostic tests for APS including the obstetric variant further stressing the key role of anti-β2GPI antibodies.

However, isolated LA is considered a risk factor even in the absence of anti-β2GPI antibodies. In this situation, there is the suggestion to look for anti-PT antibodies and in particular for anti-phosphatidylserine (PS)-PT antibodies [6].

In fact, anti-PS-PT antibodies, but not anti-PT antibodies detected with different assays, can induce the LA phenomenon and are associated with the vascular APS manifestations [6].

Therefore, their presence supports the “autoimmune” nature of the isolated LA, while the lack of both anti-β2GPI and anti-PS-PT antibodies makes the value of the isolated LA much less predictive for both the vascular and the obstetric clinical events [35, 36].

The risk profile of the obstetric APS has been recently re-evaluated. In fact, the succEssfUl pREgnancy in aPL positive women: a risK stratification Algorithm (EUREKA) study showed that also low aPL titers confer risk for pregnancy morbidity in addition to the widely accepted risk associated with medium/high aPL titers. In particular, the persistent double positivity for LA and low titers of anti-β2GPI IgG display a higher risk than low aCL and anti-β2GPI IgG, either alone or associated [37]. The presence of extensive deposits of β2GPI at the placental level may explain why even low β2GPI-dependent aPL titers can be sufficient to trigger tissue damage. This is not the case with the endothelium which does not display β2GPI unless stimulated by a perturbing agent [25].

Anti-β2GPI antibodies are definitely key players in the pathogenesis of the obstetric APS; however, modifications of the β2GPI itself could be an additional variant responsible for different clinical manifestations in women with similar aPL profiles. Ad-hoc clinical and biological studies are needed to investigate this issue.

Conclusions and take-home messages

1. APS is among the most frequent acquired-risk factors for treatable recurrent pregnancy loss.

2. aPL are auto-antibodies against PL-binding proteins and react with two major auto-antigens: β2GPI and PT. However, there is strong evidence that β2GPI is the main antigenic target for aPL responsible for the positivity for all three formal classification laboratory tests.

3. aPL detectable by LA, β2GPI-dependent aCL and anti-β2GPI assays are the formal classification laboratory tests and are currently used as diagnostic tools as well.

4. β2GPI-dependent aPL are widely accepted as pathogenic antibodies in addition of being classification/diagnostic tools.

5. Thrombotic events are not the main pathogenic mechanisms mediated by β2GPI-dependent aPL responsible for pregnancy complications.

6. β2GPI is physiologically present on the decidual endothelium, on villous and extravillous trophoblasts, and can be available for maternal aPL.

7. β2GPI-dependent aPL, once bound to decidual cells and extravillous trophoblasts β2GPI, affect trophoblast proliferation and differentiation and induce apoptosis. The same antibodies interfere with angiogenesis and spiral artery development and induce local inflammatory responses.

8. The effect of β2GPI-dependent aPL on trophoblast growth and differentiation may explain early miscarriages. On the other hand, late obstetrical manifestations can be also related to the aPL effect on placenta vasculature, including spiral arteries remodeling.

9. The persistent presence of LA or double (any combination of LA, β2GPI-dependent aCL, or anti-β2GPI antibodies) or triple (all three subtypes) aPL positivity or the presence of persistently high aPL titers defines the high-risk profile for obstetric APS according to EULAR.

10. Recent evidence supports the predictive value for pregnancy complications of persistent low aPL titers consistent with the physiological high presence of β2GPI at the placenta level.

11. β2GPI is a pleiotropic molecule whose physiological function is still elusive. The molecule was originally described as a natural anticoagulant, and its neutralization by the autoantibodies was responsible for a procoagulant state.

12. The demonstration that thrombotic occlusions are not crucial for APS pregnancy complications and the finding that β2GPI may be involved in physiological placentation suggests additional pathogenic mechanisms non necessarily linked to the reactivity with the auto-antibodies.

Abbreviations

aCL: anti-beta 2 glycoprotein I-dependent cardiolipin

aPL: antiphospholipid antibodies

APOH: apolipoprotein H

APS: antiphospholipid syndrome

IgG: immunoglobulin G

LA: lupus anticoagulant

PL: phospholipid

PS: phosphatidylserine

PT: prothrombin

β2GPI: beta 2 glycoprotein I

Declarations

Author contributions

MPL, GC and TF contributed conception and design of the review; GC organized the database; MPL wrote the first draft of the manuscript; GC wrote one section of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Not applicable.

Consent to participate

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Consent to publication

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