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An insight into the pathophysiology of thrombosis in antiphospholipid syndrome

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1. ABSTRACT

The antiphospholipid syndrome (APS) is a disorder which is characterized by the presence of autoimmune antiphospholipid antibodies (APL) and increased risk of thrombosis and fetal loss. APL are associated with recurrent abortions in APS patients and participate in the pathogenesis of venous or arterial thrombosis, although the underlying mechanisms are poorly understood. Antigens that are targeted by APL include beta 2 glycoprotein I and prothrombin. Pathological mechanisms of APL encompass inhibition of natural anticoagulants (protein C system, tissue factor pathway inhibitor, and annexin A5), inhibition of the fibrinolytic system, activation of endothelial cells, monocytes and platelets, and complement activation. In this review, we discuss the main targets of APL and prothrombogenic mechanisms of APL.

2. INTRODUCTION

Antiphospholipid antibodies (APL) participate in the pathogenesis of venous or arterial thrombosis (1-3) as well as in recurrent abortions (4, 5). These autoantibodies constitute a major marker of a clinical disorder known as antiphospholipid syndrome (APS) (6, 7). APS can occur in patients without any evidence of an associated disease (primary APS), but also in association with systemic lupus erythematosus (SLE), arthritis and other autoimmune disorders (secondary APS) (8, 9). The previous terms primary and secondary APS are now not recommended according to a very recent update of the APS criteria (10). From a laboratory standpoint, APL are mainly detected as anticardiolipin (aCL) and lupus anticoagulant (LA) antibodies (5, 11, 12). However, APL displaying similar immunoreactivities can also be found in normal subjects (2-4%), but at a very low concentration (1, 13-15).

Table 1. Most common immunoreactivities displayed by autoimmune antiphospholipid antibodies

Beta 2 Glycoprotein I
Prothrombin
• Natural anticoagulants
• System of the protein C
• Annexin A5
• Pathway inhibitor
Fibrinolytic system
FXII, FXI, FVII, Prekallikrein, Low molecular weight-kininogen, component C4

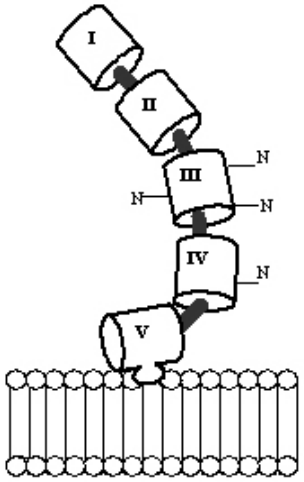

Figure 1. Schematic of the structure of beta 2 GPI, depicting its five polypetide domains (I-V), 3 of which have glicosylation sites.

3. ANTIGENS TARGETED BY APL

Antigens targeted by autoimmune APL can be grouped in four classes (Table 1): 1) main antigens: beta 2 glycoprotein I (beta 2 GPI) (16), and prothrombin (17); 2) natural anticoagulants: members of the protein C system (18), annexin A5 (19), and tissue factor pathway inhibitor (TFPI) (20); 3) proteins of the fibrinolytic system: tissue plasminogen activator (tPA) (21), plasmin (22); and 4) other proteins: factor XII, factor XI (23), factor VII (24) and high-molecular weight kininogen (HMWK) (25), among others. This section discusses the first three classes.

3.1. Main antigens

3.1.1. Beta 2 glycoprotein I

Beta 2 GPI, which is also known as apolipoprotein H, is a glycoprotein of 50 kDa formed by a single polypeptide chain of 326 amino acids with 5 domains. The first four domains (I to IV) contain approximately 60 amino acids and domain V encloses 80 aminoacids. Beta 2 GPI has 4 glycosylation sites in domains III and IV (Figure 1). The amino acidic sequence Ser311-Lys317 in domain V mediates the binding to membranes (26, 27).

Beta 2 GPI is mainly synthesized in the liver, reaching plasma concentrations of around 200 microg/ml (28). It displays a marked affinity towards negatively-charged molecules such as anionic phospholipids, heparin, lipoproteins and also to activated platelets (26).

3.1.2. Prothrombin

Prothrombin is a vitamin K-dependent glycoprotein of 72 kDa (579 amino acid) (29) synthesized in the liver which reaches an average plasma concentration of 100 microg/ml. In the N-terminal region it contains 10 glutamine residues (Gla domain) that suffer a process of gamma-carboxylation by a post-ribosomal reaction. This modification is essential for calcium (Ca^{2+})-dependent binding to phosphatidylserine (29). The peptide sequence between amino acids 40 and 270 contains two Kringle domains that are important to form a molecular complex with FVa (30). Under physiological conditions, prothrombin bound to anionic phospholipids is activated by the prothrombinase complex, generating thrombin by the cleavage of two peptide bonds (Arg322-Ile323 and Arg271-Thr272). Thrombin, among other actions, converts fibrinogen into fibrin, and also forms a complex with thrombomodulin on the endothelial cell (EC) surface to activate protein C, which in turn inactivates FVa and FVIIIa (30).

3.2. Natural anticoagulants

3.2.1. Protein C

PC is a vitamin K-dependent glycoprotein secreted by the liver (31). To perform its anticoagulant function, active PC (APC) is generated on the surface of ECs, where it forms a high affinity and reversible complex with thrombin, thrombomodulin, the cofactor protein S (PS) and calcium (31, 32). Once activated, APC binds to its cofactor PS and exerts its anticoagulant activity on the phospholipid surface by inactivating FVa and FVIIIa. The inhibition of both PC activation and APC function has been observed in the APS (33).

3.2.2. Tissue factor pathway inhibitor

TFPI, which is present in ECs, platelets and in circulation, binds to FXa in the presence of Ca^{2+}. The TFPI/FXa complex interacts with FVIIa/TF, rendering FVIIa inactive through an interaction between the TFPI second domain and FVIIa. It is plausible that deficiencies and/or defects in any of these proteins may produce a serious imbalance of hemostatic mechanisms and therefore increase the risk of developing thrombotic events.

3.2.3. Annexin A5

Annexin A5 is a 35 kDa (34) protein expressed in syncytiotrophoblast and ECs (35) which has a structural shape resembling a concave disc. It binds to anionic phospholipids in a Ca^{2+}-dependent manner, and plays an important role in preventing clotting reactions on the platelet surface (36).

3.3. Proteins of the fibrinolytic system

The fibrinolytic system is composed by activators and inhibitors that regulate the conversion of the circulating proenzyme, plasminogen (Pg), into the active enzyme plasmin, which produces the lysis of fibrin (30). Additional components of the fibrinolytic system include Pg activators

(tissue and urokinase type), Pg activator inhibitor 1 and 2 (PAI-1 and PAI-2), plasmin and alpha$_2$ antiplasmin (37).

4. CHARACTERISTICS OF APL

4.1 Hypothesis for APL formation

Different mechanisms have been proposed to explain autoantibody production in APS (38): a) Relationship between beta 2 GPI, apoptotic cells and phosphatidylserine (PS): In addition to PS, apoptotic cells express on their surface apoptotic blebs which contain intracellular debris (39), and these antibodies increase the affinity of beta 2 GPI for PS by 1000 fold (40); b) Apoptotic cell clearance: it has been suggested that beta 2 GPI (41, 42) and prothrombin (43) in APS may bind to expose PS on apoptotic blebs. Impairment in apoptotic cell clearance, or an increase in apoptotic cell generation may predispose an individual for developing antibodies directed against beta 2 GPI, prothrombin and annexin A2 bound to the apoptotic blebs (39); c) Autoreactive CD4+-T cells: autoreactive T cells against beta 2 GPI have been identified in patients with APS (44). It was hypothesized that in states associated with impaired clearance of apoptotic cells, beta 2 GPI bound to surface exposed PS on apoptotic cell may provide substrate for the presentation of cryptic epitopes (44); d) Toll-like receptor: oxidized beta 2 GPI is able to bind to dendritic cells and induce their maturation. Dendritic cell activation leads to a signalling cascade resembling that triggered by a toll-like receptor family 4 (TLR4) (45). This raises the possibility that oxidized beta 2 GPI alone, or in complex with anti-beta 2 GPI antibodies, may act as an endogenous immunological adjuvant driving additional antibody generation via TLR4 (45); and e) Molecular mimicry: it has been suggested that anti- beta 2 GPI antibodies may be generated as a result of molecular mimicry between human beta 2 GPI and molecules similar to beta 2 GPI in invading bacteria (46). This has been observed in mice immunized with Haemophilus influenzae, Neisseria gonorrhoeae and tetanus toxoid, which developed anti-beta 2 GPI antibodies (46).

4.2. Anti-beta 2 glycoprotein antibodies

Anti-beta 2 GPI antibodies are part of a heterogeneous group of immunoglobulins that differ in their biological activities. They are directed against different epitopes located throughout the five domains of beta 2 GPI (47). The affinity of beta 2 GPI to phospholipid surfaces is low, but increases 100-fold in the presence of anti-beta 2 GPI antibodies. On the other hand, anti-beta 2 GPI antibodies have low affinity in solution, a fact that could explain the absence of immune complexes in the serum of APS patients (48).

4.3. Anti-prothrombin antibodies

In the presence of Ca^{+2}, anti-prothrombin antibodies bind bivalently and with low affinity to prothrombin associated with anionic phospholipids. This interaction is similar to that described for anti-beta 2 GPI antibodies (48). Although studied in less detail that the antigenic specificity of anti-beta 2 GPI antibodies, it is known that anti-prothrombin antibodies bind prethrombin (carboxy terminal segment of prothrombin), as well as fragments 1 and 1.2 belonging to the amino terminal domain of prothrombin (49, 50). Most of the antibodies are directed against the first two-mentioned segments. The anti-prothrombin antibodies have anticoagulant and procoagulant activity based on their ability to interfere with the action of the prothrombinase complex, and to compete for a lipid surface, respectively (48).

4.4. Antibodies against natural anticoagulants

It is likely that beta 2 GPI exerts a physiological inhibition favoring the binding of PS to C4bp, a protein of the complement system; and thus anti-beta 2 GPI antibodies might hinder this function. In the absence of free PS, PC would not be activated, and therefore FVa remains active for a longer time favoring fibrin formation and the development of a prothrombotic state (51, 52).

Alterations in the TF pathway are associated to hypercoagulable states in APS (53). In this sense it is noteworthy that anti-TFPI antibodies have been frequently detected in APS patients (20, 54, 55).

APL and annexin A5 bind to phospholipids, and it is likely that antibodies compete for the same site. Remarkably, low levels of annexin A5 have been reported in syncytiotrophoblasts of APS patients (56). Similar findings were reported when placentas from normal subjects were incubated with IgGs from APS patients (56).

4.5. Antibodies against fibrinolytic components

The presence of antibodies against tissue-type Pg activator (t-PA, anti-tPA) has been demonstrated in patients with APS (49). Some of these antibodies bind to the catalytic domain of the t-PA. There is an inverse correlation between the activity of t-PA and levels of anti-t-PA antibodies, a condition that produces hypofibrinolysis (21).

5. CHARACTERISTICS AND CONSEQUENCES OF THE ANTIGEN-ANTIBODY INTERACTION

APL have been associated to thrombosis through the following mechanisms: (a) inhibition of the natural anticoagulants and the fibrinolytic system, and (b) cellular activation.

5.1. Alterations in the coagulation and fibrynolitic systems

The increased affinity of beta 2 GPI for phospholipid surfaces in the presence of anti-beta 2 GPI antibodies may modify the physiological function of beta 2 GPI and affect the coagulation/fibrinolysis balance on the cell surface by interacting with other phospholipids-binding proteins, such as coagulation factors and several components of the PC system (57).

Different mouse model have been developed that develop aCL antibodies that are beta 2 GPI-dependent and direct anti-beta 2 GPI antibodies, thus offering a good model for studying APS (58, 59). Using a homologous recombination approach, Miyakis *et al* (60) generated knock-out mice lacking the beta 2 GPI gene, which are fertile but exhibit moderately compromised early pregnancy. Notably, these mice exhibit decreased ability for thrombin generation *in vitro*. On the other hand, immunization of atherosclerosis-prone LDL receptor (LDLR) knockout mice with human

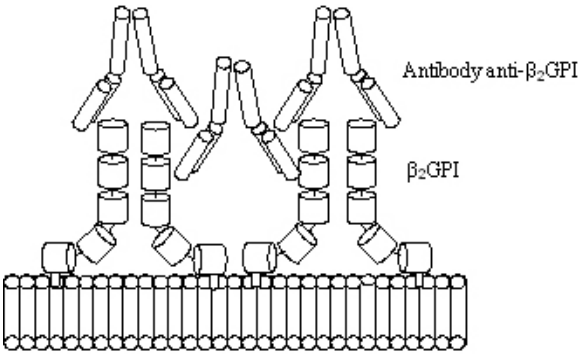

Figure 2. Formation of immune complexes between beta 2 GPI and antiphospholipid antibodies. Two molecules of beta 2 GPI and one molecule of antibody form a stable trimolecular complex, which can interfere with several phospholipids-dependent hemostasis reactions.

aCL antibodies from an APS patient caused an acceleration of atheroma formation, thus providing additional evidence for a causal pathogenic effect (61).

Two molecules of beta 2 GPI and one of anti-beta 2 GPI are necessary for the formation of a bivalent immune complex on the phospholipid layer (48). This interaction originates a dynamic structure because the ternary complex can be dissociated into a bimolecular complex, which could then be released from the lipid membrane. The formation of the bimolecular complex, the binding to phospholipid membranes and its further dissociation, is a slow process that allows other proteins to have access to the lipid surface. The dynamic of the complex formed by a mixture of IgG directed against different epitopes of beta 2 GPI, as it occurs in APS patients, is quite different because of the formation of immune complexes which are interconnected on the lipid surface (Figure 2) (48). Such complexes impede the contact of other proteins with the lipid surface, interfering with some phospholipid-dependent reactions of hemostasis, such as activation of protein C. This in turn leads to the assembly of the complex tenase-prothrombinase, the activation of contact system factors, and the formation of the TF/FVII complex. A similar mechanism has been reported for the bivalent immune complex formed by two prothrombin molecules, antibodies anti-prothrombin and calcium, thus interfering with clotting reactions (48).

The procoagulant activity of anti-prothrombin antibodies is based on: a) increased binding of prothrombin to the surface of anionic phospholipids without interfering with the action of the prothrombinase complex, which then favors thrombin formation (62); b) antithrombin interference is caused by the binding of anti-prothrombin antibodies which are found near the thrombin binding site (63); and c) the presence of homologous amino acid sequences in thrombin and plasmin, which could be potential epitopes for anti-prothrombin antibodies on plasmin, which may then interfere with the action of the plasmin to dissolve the fibrin layer that forms the clot (64).

There is evidence showing that clotting is markedly increased by removing annexin A5, which leaves the apical membrane exposed to blood flow (65). Similar result is observed when ECs are incubated with anti-annexin A5 antibodies (65). It is important to identify the mechanisms by which APS causes placental thrombosis and fetal loss. Of note in this regard, annexin A5-null deficiency in the mouse did not have an impact on litter size and fetus viability (36).

Changes in the tissue factor (TF) pathway of coagulation have been implicated in the development of hypercoagulability in APS (53). Indeed, inhibitory activity (in IgG fractions) against TFPI (55) and autoantibodies (in plasma) to TFPI (54) have been identified in APS subjects. Adams *et al* (20) found anti-TFPI activity in 65% of APS subjects, and IgG fractions from these patients showed an interference with the TFPI system and induced thrombin generation, a phenomenon that could explain the increased thrombotic risk in APS patients. Disruption of the TFPI gene was not compatible with normal embryonic development in mice (66), whereas severe immunodepletion of TFPI in a rabbit model dramatically altered the threshold by which tissue factor may activate coagulation (67, 68).

The presence in APS patients of antibodies directed to phospholipid binding proteins, such as anti-PC, anti-PS and anti-thrombomodulin antibodies, may contribute to thromboembolic complications (69). The assembly of coagulation complexes on negatively-charged phospholipids surfaces is a prerequisite for their activity. Given that these antibodies compete with phospholipid binding proteins for the catalytic surface, it is logical to assume that they may also inhibit the binding of PC and PS and thereby their activity (69, 70).

Endothelial PC/APC receptor (EPCR) is an EC membrane glycoprotein that is tightly bound to phospholipids and binds PC and APC (71). PC binding to EPCR notably enhances its activation by the thrombin-thrombomodulin complex (72). The analysis of EPCR knockout mice supports a role for EPCR in pregnancy maintenance, since these animals exhibit placental thrombosis and early embryonic mortality (73). Hurtado *et al* (74) had shown that anti-EPCR autoantibodies constitute a risk factor for a first fetal death episode in APS.

With respect to effects on the proteins of the fibrynolitic system, plasmin produces a cleavage in the V domain of beta 2 GPI, particularly between Lys317-Thr318, as FXa does, but less effectively. This cleavage produces a diminished affinity towards phospholipids and a major reactivity to plasminogen and, as a consequence, decreases plasmin generation. However, the concentration of beta 2 GPI needed to inhibit fibrinolysis is higher (>0.25μM) than its average level in plasma (0.01 μM). Heparin accelerates the proteolytic cleavage of beta 2 GPI by plasmin, and it is likely that high concentrations of altered beta 2 GPI may locally produce hypofibrinolysis (51, 52, 75).

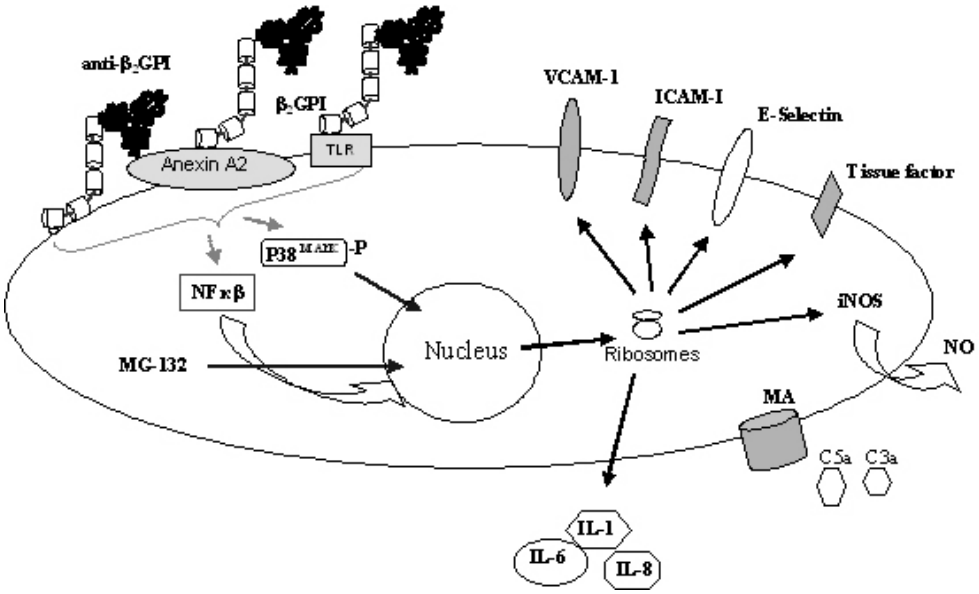

Figure 3. Activation of endothelial cells by antiphospholipid antibodies. NO, nitric oxide; iNOS, inducible nitric oxide synthase; MAC, membrane attack complex; MG-132, proteasome inhibitor.

5.2. Effects of APL on different cells

In this section we discuss the pathophysiological role of APL on ECs, platelet and monocyte activation.

5.2.1. Activation of endothelial cells

The presence of thrombophilic vascular events in patients with APS suggest a putative reactivity of APL with ECs (Figure 3) (76). Such reactivity seems to be secondary to the binding of anti-beta 2 GPI antibodies to beta 2 GPI present on the EC membrane. The possibility that anti-beta 2 GPI antibodies activate ECs by the interaction with some molecules that trigger an inflammatory response may explain the apparent paradox raised in annexin A2 studies (77).

Both anti-beta 2 GPI and anti-annexin A2 bivalent antibodies can induce activation of ECs. Annexin A2 bound to the external EC membrane can interact with anti-annexin A2 antibodies. Alternatively, the anti-beta 2 GPI-beta 2 GPI complex may also bind to annexin A2. This interaction may, in turn, promote the aggregation of the membrane adapter protein associated to annexin A2.

The myeloid differentiation protein (MyD88), which drives NFκB activation produced by Toll-Like receptors (TLRs) (77), has been suggested to be involved in the cellular activation mediated by the binding of anti-beta 2 GPI antibodies.

EC activation by anti-beta 2 GPI induces the expression of adhesion molecules and the secretion of cytokines secretion, such as those produced by incubating ECs with proinflammatory agonists like interleukin 1 (IL-1), tumor necrosis factor alpha (TNF-alpha) and lipopolysaccharide (LPS) (78) (Figure 3). These effects are mediated by NFκB activation. Augmented expression of adhesion molecules in ECs of mice with APL occurs with a concomitant increase of leukocyte adhesion (79).

It has been demonstrated in human umbilical vein endothelial cells (HUVECs) that APL enhance the expression of several adhesion molecules (intercellular cell adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1] and E-selectin), as well as monocyte adherence to ECs *in vitro* (80). Two studies have indicated that the levels of soluble ICAM-1 and VCAM-1 were significantly increased in the plasma of patients with APS, which supports the hypothesis that APL antibodies activate ECs and may create a hypercoagulable state in APS patients (81, 82). Remarkably, by analyzing the effects of APL on leucocytes obtained from mice singly deficient for ICAM-1 and doubly deficient for ICAM-1/P-selectin, Pirangeli *et al* (79) concluded that ICAM-1, P-selectin, and VCAM-1 expression are important in thrombotic complications by aPL antibodies and may provide novel targets for therapy in patients with APS.

The interaction of APL with ECs has also important consequences on nitric oxide production, as demonstrated in mice treated with anti-beta 2 GPI antibodies (83), as well as by the decrease observed in nitric oxide–dependent vasorelaxation in brachial artery of patients with APS, as assessed by ultrasonography (84).

During activation or apoptosis of EC, microparticles (MP) are released from membranes (79). These vesicles are highly procoagulant, as they contain anionic phospholipids and receptors for blood coagulation factors. In addition to this procoagulant potential, MP are able to trigger activation and adhesion of different cellular types. High number of MP has also been reported in the serum of patients with APS (2).

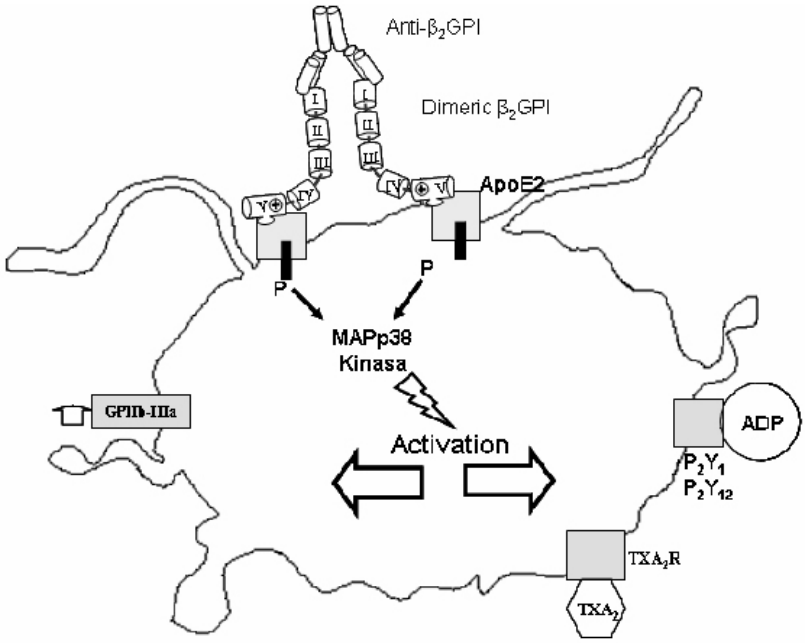

Figure 4. Platelet activation by antiphospholipid antibodies. P_2Y_1 and P_2Y_{12}: ADP receptors; TXA_2: thromboxane A_2; TXA_2R: thromboxane A_2 receptor.

5.2.2. Platelet activation

The association of beta2 GPI dimer with the platelet membrane requires its interaction with the apoER 2 receptor, a member of the LDLR family present in platelets (Figure 4). Some studies have demonstrated that beta 2 GPI can bind other members of the LDLR, such as VLDLR. At present, the capacity of beta 2 GPI dimer to interact with LDLR family members is well recognized (85).

The engagement of dimer receptors at the platelet surface triggers a cascade of phosphorylation reactions involving members of the MAP kinase family, such as MAPKp38, resulting in increased synthesis of tromboxane A2, a potent vasoconstrictor and platelet agonist (86). In individuals with APS, the presence of an imbalance in TXA2/prostacyclin ratio could favor the development of thrombosis (51, 52, 87).

Platelet activation by APL also produces an increase in the expression of both CD63 (88, 89) and GPIIb-IIIa (90, 91), as well as the release of P-selectin (CD62P) (2), and platelet-derived MP (2).

5.2.3. Monocyte activation

TF is the physiological initiator of physiological coagulation as well as clotting observed in thrombotic disease. TF is a high-affinity receptor for coagulation factor VIIa and functions as an essential cofactor for factor VIIa to efficiently cleave factors IX and X to their active forms (factors IXa and Xa, respectively). Cell-bound TF is a 47-kDa transmembrane glycoprotein that is constitutively expressed on the surface of various cell types outside the vasculature, but it is not expressed on ECs or peripheral blood cells (at least not in a functionally active form) (92).

ECs, blood monocytes, and other cells in contact with flowing blood do not constitutively express functional TF and do not have intracellular stores of TF (93). However, these cells express TF activity via transcription and synthesis of nascent TF molecules in response to stimulation with certain agents, including lipopolysaccharide (LPS) (94), endothelial MP (95), chemokines (96), anti-platelet factor 4/heparin antibodies (97), homocysteine (98), P-selectin (99) and/or certain inflammatory cytokines. There is a growing body of evidence suggesting that hypercoagulability in APS patients is instigated, at least in part, by an increase in TF activity in circulating blood monocytes caused by autoantibodies. Different groups have found that serum, plasma, purified total IgG, and anti-beta 2 GPI antibodies from APS patients enhance TF expression and procoagulant activity on normal monocytes (53, 100-105). $F(ab)_2$ antibody fragments retain these procoagulant effects, suggesting that Fc receptors are not required for the procoagulant activity of these antibodies (100, 106). Additionally, several of these studies have demonstrated that monocytes isolated from APS patients exhibit an increased expression of TF protein and mRNA (53, 105, 107, 108), and that anti-beta 2 GPI human monoclonal antibodies derived from peripheral B cells of APS patients enhance monocyte's TF activity and levels of TF mRNA in a beta 2 GPI -dependent manner (53, 103). On the other hand, it has been reported that beta 2 GPI-specific T cells are of the TH_1 phenotype and produce interferon-gamma, a cytokine known to stimulate TF expression in monocytes (109, 110).

5.3. Activation of the complement system

Several studies have suggested that complement activation by APL mediates fetal loss and thrombosis in

APS patients (111-114). Using specific complement inhibitors or mice deficient in several complement components, Girardi *et al* (111, 113) has shown that C4, C3, C5 and C5a-C5aR are required to induce fetal injury by APL. Furthermore, Pierangeli *et al* (114) showed that mice deficient in complement C3 and C5 are resistant to thrombosis, EC activation and fetal loss induced by APL (114). They propose that pathogenic APL, in addition to their direct effects on platelet and EC targets, induce complement activation, and thus generate complement split products that attract inflammatory cells and initiate thrombosis and tissue injury. The blockade of C5 is effective in preventing thrombosis in a mouse model of APS, suggesting that complement activation may be a valuable target for interventions that prevent, arrest or modify the thrombogenic effects of APL.

A proposed mechanism for APL-induced fetal damage is that when these antibodies act on the placenta they generate C5a, which attracts and activates neutrophils and monocytes and stimulates the release of inflammatory mediators and other molecules, such as proteolytic enzymes, chemokines, cytokines, C3 and properdin. Neutrophils have been implicated in pregnancy loss in an antibody-independent form, and C5a could enhance this effect in APS (113, 115).

On the other hand, some data suggests that complement activation mediates two important effects of APL, induction of thrombosis and activation of ECs (114). This notion is supported by the observation that human polyclonal immunoglobulin G with anti-beta 2 GPI activity triggers clotting in the microcirculation of rat mesentery in the presence of a priming proinflammatory stimulus, and that the terminal complement complex is required for APL-dependent thrombus formation (116). Moreover, it has been reported high frequency of complement-fixing activity of aCL in APS (117).

Given the participation of the complement system in thrombosis and fetal loss, it is tempting to speculate that the inhibition of complement activation may be beneficial for the treatment of thrombosis and pregnancy complications in women with APS. Interestingly, heparin, the standard anticoagulant that is used in obstetric patients, has anticoagulant activity and capacity to block complement activation (118).

6. CONCLUSIONS

Autoimmune APL participate in the pathogenesis of the APS. Several antigens are targeted by APL, including beta 2 GPI, prothrombin and others proteins. The main mechanisms involved in thrombosis associated with APL are: 1) inhibition of natural anticoagulants; 2) inhibition of the fibrinolytic system; 3) cellular activation; and 4) complement activation. Further understanding of the relationship of APL with the haemostatic system may facilitate the development of new therapeutic strategies targeted to more specific mechanism of thrombosis in APS.

7. ACKNOWLEDGEMENTS

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Abbreviations: aCL: anticardiolipin antibodies; APC: activated protein C; APL: antiphospholipid antibodies; APS: antiphospholipid syndrome; beta 2 GPI: beta 2 glycoprotein I; EC: endothelial cell; EPCR: endothelial PC/APC; HMWK: high-molecular weight kininogen; ICAM-1: intercellular cell adhesion molecule-1; IL-1: interleukin 1; LA: lupus anticoagulant; LPS: lipopolysaccharide; MP: microparticles; PAI-1 and PAI-2: plasminogen activator inhibitor 1 and 2; PC: protein C; Pg: plasminogen; PS: protein S; SLE: systemic lupus erythematosus; TF: tissue factor; TFPI: tissue factor pathway inhibitor; TLR4: toll-like receptor family 4; TNF-alpha: tumor necrosis factor alpha; tPA: tissue plasminogen activator; VCAM-1: vascular cell adhesion molecule-1.

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