Mechanisms of Action of the Antiphospholipid Antibodies

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3.1 INTRODUCTION

Antiphospholipid syndrome (APS) is a chronic autoimmune condition clinically characterized by vascular thrombosis and/or pregnancy complications. The laboratory diagnosis of APS relies on the identification of circulating antiphospholipid antibodies (aPL) using three tests: two solid-phase assays detecting anticardiolipin (aCL) and anti-\(\beta_2\) glycoprotein I antibodies (anti-\(\beta_2\)GPI), plus the functional assay lupus anticoagulant (LA) \cite{1}. Medium/high-titre aPL positivity, confirmed 12 weeks apart, of at least one test is required to diagnose APS \cite{1}. In addition, aPL not only provide diagnostic biomarkers of APS, but also exert a pathogenic role (Fig. 3.1). aPL with the same autoantigen specificity and titres can be related to different clinical pictures and pathogenic mechanisms in experimental models \cite{2}; each aPL profile might associate with merely vascular events or pregnancy complications \cite{1}. These observations explain why aPL are regarded as a risk-factor for APS, with additional hits required to trigger clinical manifestations \cite{2}. Such cofactors might account for the clinical and biological disparity between vascular and obstetric variants of the syndrome.

3.2 ANTIPHOSPHOLIPID ANTIBODIES

aPL are a heterogeneous family of autoantibodies, but there is evidence that only antibodies reacting with phospholipid (PL)-binding proteins, particularly \(\beta_2\) glycoprotein I (\(\beta_2\)GPI) and prothrombin (PT), display a pathogenic potential.
3.2.1 The β2 Glycoprotein I-Dependent Autoantibodies

β2GPI is a single-chain 43-kDa glycoprotein synthesized by endothelial cells (EC), hepatocytes, and trophoblast cells. A member of complement control protein (CCP) family, β2GPI consists of 326 amino acids arranged in five CCP-repeat domains (D). DI-IV comprise 60 amino acids and contain two disulphide bridges each; DV is aberrant, consisting of 82 amino acids crosslinked by an additional disulphide bond. DV is responsible for binding to PL and cell membranes. Three β2GPI configurations have been described: (1) a circular form, adopted by circulating plasma β2GPI, (2) a J-shaped configuration, assumed upon binding to anionic surfaces, such as cardiolipin (CL) and other PL or lipopolysaccharide (LPS), and (3) an intermediate S-shape. β2GPI interacts specifically with LPS through the C-terminal, potentially acting as an LPS carrier or scavenger [3].
aPL reacting with β2GPI are currently regarded as the main antibody subset. Affinity-purified anti-β2GPI IgG trigger a pathogenic effect in all in vivo models, while specific absorption of anti-β2GPI activity inhibits the thrombotic effect [4]. Antibodies against β2GPI are the main mediators of LA, an in vitro elongation of PL-dependent clotting time. High-titre antibodies, often of IgG isotype, mediate this functional phenomenon. Anti-β2GPI antibodies partially overlap with autoantibodies identified with aCL enzyme-linked immunosorbent assay (ELISA) test, which can employ CL-coated matrix and bovine or human serum, thus detecting antibodies against β2GPI-bound CL (β2GPI-dependent aCL) or CL alone (β2GPI-independent aCL).

A positively charged discontinuous structure in β2GPI-DI is the main epitope involved in β2GPI/anti-β2GPI antibody binding. This structure is cryptic and conformation-dependent, available for antibody binding only when β2GPI opens to a J-configuration. In the circular conformation, DI interacts with DV, thus hiding the critical epitope [5]. The immunogenicity of β2GPI also depends on its conformation, as supported by in vivo evidence: mice develop antibodies against DI only when injected with misfolded β2GPI or β2GPI-CL [6].

Anti-DI antibodies can be detected in most APS patients, and are significantly associated with LA. The pathogenicity of such autoantibody subset has been progressively characterized: infusion of a synthetic DI peptide partially protects naïve mice from the thrombogenic effects of polyclonal aPL IgG [7]. A direct demonstration of their pathogenic effect was obtained using MBB2, a human monoclonal IgG antibody targeting β2GPI-DI. Its infusion induces foetal losses in pregnant mice and blood clots in rats after LPS priming [8]. Further support comes from tolerogenic dendritic cells (tDCs) pulsed with the whole molecule or β2GPI-DI: infusion of tDCs to β2GPI-immunized BALB/c mice results in reduced foetal loss rate, decreased anti-β2GPI antibody titres, and raised expression of anti-inflammatory cytokines. A greater effect is obtained with DC pulsed with DI than the whole molecule [9].

It could be concluded that anti-DI are the pathogenic antibodies, and anti-DIV/V are innocent players because IgG reacting with β2GPI from asymptomatic carriers preferentially recognize DIV/V epitopes. However, the real scenario is much more complicated: approximately one third of APS patients carrying anti-β2GPI antibodies are negative for anti-DI IgG [10,11]. A multi-centre study exploiting several peptides spanning the different domains confirms that a consistent rate of APS patients display autoantibodies reacting with β2GPI epitopes other than DI [12].

### 3.2.2 Prothrombin-Dependent Antibodies

PT (factor (F) II) is a 72-kDa vitamin K-dependent glycoprotein synthesized in the liver. PT physiological activation is mediated by the prothrombinase complex, which enlists activated FX, FV, calcium, and PL. Prothrombinase complex converts PT into thrombin only when negatively charged PL bind to
PT. To be antigenically recognized, human PT has to be coated on activated plates or exposed to immobilized phosphatidylserine (PS) via calcium ions. ELISA-detecting antibodies against PS/PT complex (anti-PS/PT) identify a partially different autoantibody population from the assay using PT as the only antigen [13].

In vitro experimental findings suggest antibodies against PT exert thrombogenic effects interfering with fluid-phase coagulation components and activating EC. Together with anti-β2GPI antibodies, antibodies against PT (anti-PT) constitute the major contributor to LA phenomenon: approximately two-thirds of IgG anti-PT display in vitro anticoagulant activity. Such elongation of clotting time could be explained by PT/anti-PT/PL trimolecular complexes inhibiting the activation of prothrombinase and tenase complexes and competing with clotting factors for PL surfaces [14]. Due to the lack of cross-reactivity of human antibodies with animal PT, evidence from animal models is weak. Anti-PS/PT are more closely related with clinical events than anti-PT, suggesting that pathogenic antibodies may recognize a conformational epitope(s) expressed when PT complexes with anionic PL in the presence of calcium ions [15]. Human monoclonal or affinity-purified polyclonal anti-β2GPI antibodies from a serum reacting with both β2GPI and PS/PT react toward β2GPI only, clearing the contentious issue of the potential cross-reactivity between anti-PS/PT and anti-β2GPI antibodies [16].

### 3.2.3 Antibodies Against Other PL Antigens

The pathogenic roles of several autoantibodies that target negatively charged PL other than CL have been evaluated in APS. PS, phosphatidylinositol, and phosphatidic acid are among the best-characterized antigens. aCL are well-known to broadly cross-react with antibodies targeting both PS and phosphatidylinositol, due to the recognition of β2GPI/PL complex. Therefore, cross-reactivity is mainly mediated by autoantibodies reacting with β2GPI [17].

Antibodies targeting phosphatidylethanolamine (PE) deserve more attention. PE, a zwitterionic PL, promotes thrombosis by activating FX and PT, and works as anticoagulant potentiating activated protein C (APC) activity. Antibodies against PE (anti-PE) bind to kininogen, leading to antibody/PE/kininogen trimolecular complexes that enhance thrombin-induced platelet aggregation. An in vivo demonstration of anti-PE pathogenicity in vascular events is lacking; anti-PE infusion to pregnant mice triggers placental thrombosis and haemorrhage [18].

### 3.3 aPL-MEDIATED MECHANISMS OF THROMBOSIS

The association between aPL and thrombosis is supported by several epidemiological studies, with clot formation as the key-event [2]. Most evidence about aPL-mediated thrombus formation has been gained from in vitro models, with
further support coming from three different in vivo models of thrombosis [2]. aPL can increase the size of thrombi triggered by mechanical or chemical stimuli; in a third model, infusion of human aPL IgG with a small LPS amount is sufficient to induce clotting [19].

aPL procoagulant mechanisms are mainly mediated by antibody reactivity against PL-binding proteins on membranes of different cells. It is still unclear whether aPL significantly react with PL-binding proteins in fluid-phase. Complex formation in fluid-phase requires stoichiometric antigen–antibody ratios uncommon in patients because aPL are low-avidity antibodies [20]. Furthermore, circulating β2GPI adopts the circular form and opens upon binding to PL on the cell membrane, where the high antigenic density allows for easier autoantibodies engagement.

3.3.1 Endothelial Cells

Endothelium acts as the main player in APS pathogenesis: indeed, aPL induce a proinflammatory and procoagulant endothelial phenotype leading to clotting events.

aPL drive a significant upregulation of cellular adhesion molecules (CAMs) in in vitro EC. CAM expression on vascular surface favours leucocyte adhesion to the endothelium, contributing to APS prothrombotic diathesis. Accordingly, in vivo aPL infusion increases endothelial adhesion of leucocytes. Ex vivo studies, although inconsistently, report raised levels of soluble CAM in APS subjects. Moreover, aPL upregulate proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and IL-8 in in vitro EC, modulate vascular tone inhibiting endothelial nitric oxide synthase, and altering prostaglandin metabolism [21].

APS patients consistently display endothelial perturbation, particularly impaired brachial artery flow-mediated vasodilation response, increased circulating EC, tissue plasminogen activator (tPA), and von Willebrand factor (vWF) compared with controls [2,22].

3.3.2 Monocytes

Monocytes contribute to APS pathogenesis providing the main source of tissue factor (TF), which is the major initiator of clotting cascade. aPL significantly increase TF expression in both monocytes and EC. Vascular endothelial growth factor and its receptor Flt-1, two mediators upstream of TF, are upregulated in monocytes from APS patients [2,21].

3.3.3 Platelets

Even though aPL are well-known to induce aggregation and activation of platelets, prestimulation by agonists such as thrombin or collagen is a required step. Since PS is a negatively charged PL, it favours β2GPI adhesion on platelet
membrane with the antigen in the optimal conformation for interaction with aPL. Furthermore, aPL affect β2GPI inhibition of vWF: aPL – upon binding to β2GPI – neutralize such interaction, thus interfering with vWF-dependent platelet adhesion. This observation might explain the mild thrombocytopenia frequently observed in aPL carriers. Additional evidence of platelet activation by aPL comes from rat models: (1) in animals pretreated with low concentration of adenosine diphosphate, aPL infusion produces a platelet-rich thrombus, and (2) platelets contribute to thrombus formation induced by photochemical trauma. Ex vivo studies provide concordant findings: elevated levels of platelet-derived thromboxane metabolic products are found in urine of APS patients [2,21].

### 3.3.4 Neutrophils

Neutrophils act as additional players in coagulation: upon cell death, they release extracellular traps (NET), consisting in decondensed chromatin with nuclear proteins. NET actively participate in coagulation processes: NET-derived proteases activate the coagulation cascade and their structure serves as scaffolding for clot assembly. NET might also damage the endothelium, providing potential mediators of atherosclerosis and arterial thrombosis. NET have been recently evaluated specifically in APS: compared with healthy volunteers, sera and plasma from patients display elevated levels of both cell-free DNA and NET, APS neutrophils spontaneously release higher levels of NET. Sera and IgG from APS patients and human aPL monoclonals, especially those targeting β2GPI, stimulate NET release from control neutrophils, a mechanism abrogated by inhibitors of reactive oxygen species formation and toll-like receptor (TLR) 4 signalling [23].

### 3.3.5 Soluble Phase

The evidence of aPL interference with fluid-phase components of coagulation has been gained mostly from in vitro models and a few ex vivo experiments [2,24,25]. It has been found that aPL react against several members of serine protease (SP) family, which enlists procoagulant factors as thrombin, PT, FVIIa, FIXa, and FXa, anticoagulants such as protein C (pC) and agents involved in fibrinolysis such as plasmin and tPA. aPL interaction with these proteins is mediated by conformational epitopes shared by β2GPI and SP enzymatic domain. Importantly, aPL interaction with thrombin and FXa interferes with formation of thrombin–antithrombin (AT) and FXa-AT complexes, thus hindering AT-inactivation of thrombin and FXa. Moreover, aPL disrupt pC and protein S (pS) pathways: aPL reacting against pS or pC have been found in APS subjects, and are associated with decreased levels of pC or pS. Positivity rates of anti-pC and anti-pS antibodies in APS populations vary widely across reports, as well as their association with clinical events [26,27]. In addition, aPL decrease APC activity by competing for PL binding, an increased APC resistance has been demonstrated in APS patients.
Some aPL inhibit plasmin-mediated fibrinolysis, particularly impairing fibrin dissolution by plasmin or inhibiting tPA-mediated conversion of plasminogen to plasmin. Antibodies against tPA have been described in APS patients, inversely correlating with plasma tPA activity. Other than the inhibitory effects on anticoagulants, aPL may increase the enzymatic activity of procoagulants: some aPL subsets induce a gain-of-function of PT, which leads to increased fibrin production. Furthermore, aPL disrupt the crystallization on EC of AnnexinA5, a potent anticoagulant that prevents PL bioavailability for coagulation enzymes [2,24,25].

3.3.6 Complement

Complement activation provides a necessary step in aPL-mediated thrombosis. Most APS sera fix complement in vitro; however, the strongest evidence of complement role in aPL thrombotic events pertains to in vivo models. Animals deficient in complement components or complement receptors or treated with inhibitors of complement activation are protected from aPL thrombogenic effects. The involvement of complement cascade is further confirmed by the efficacy of the monoclonal anti-DI MBB2 and the failure of the parent monoclonal antibody MBB2ΔCH2 to induce vascular thrombosis in rats [8]. MBB2ΔCH2, which displays the same antigen specificity of MBB2 but does not activate complement because it lacks the CH2 domain, prevents aPL procoagulant effects in vivo by competing with autoantibodies for binding to β2GPI [8]. Indirect evidence also comes from the in vivo effectiveness of complement C5-inhibitor rEV576 coversin, which inhibits aPL-mediated venous thrombosis and TF production in a mouse model [28]. However, a clear decrease of complement levels has not been described in patients, only two studies report mild hypocomplementemia in primary APS [2].

3.4 aPL-MEDIATED MECHANISM OF PREGNANCY COMPLICATIONS

aPL provide the most frequently acquired risk-factor for pregnancy complications [1]. This association is clearly supported by experimental models: passive transfer of aPL IgG induces foetal loss and growth retardation in pregnant naive mice [2]. The placental tropism of aPL could be explained by the high β2GPI amount found on trophoblast: β2GPI binds to PS on external membranes of trophoblast undergoing syncytium formation. Since the earliest histopathological report of APS placentas, aPL have been thought to induce spiral artery thrombosis leading to placental infarction, and the resulting impairment of maternal–foetal blood exchange was believed to interfere with pregnancy physiology [29]. Surely aPL can induce a procoagulant state disrupting the anticoagulant AnnexinA5 shield on trophoblast. A reduced amount of AnnexinA5 consistently covers the intervillous surfaces in placentas of aPL-positive women.
However, histopathological studies report a similar prevalence of intervillous thrombosis in aPL-positive and aPL-negative women; most miscarriage samples and placentas from APS patients do not display any histopathological finding that suggest thrombosis [30]. Placental thrombosis and infarction are unlikely causes of early loss since a significant maternal blood flow does not occur in intervillous spaces until the end of the first trimester. It is increasingly acknowledged that nonthrombotic mechanisms might be implicated in APS-associated pregnancy complications [29]. This is the case of placental inflammation. In humans, aPL can induce first-trimester trophoblasts, a potent proinflammatory cytokine, IL-1β, via the inflammasome. Injections of large amounts of human aPL to pregnant naive mice after embryo implantation elicit strong placental inflammatory damage that results in foetal resorption and growth retardation. Immunohistochemical and histological examinations of decidua show neutrophil infiltration and local tumour necrosis factor (TNF)−α secretion, with transient increase in blood TNFα. Mice deficient in D6, a placental receptor that targets to degradation inflammatory chemokines, are more susceptible to foetal loss when infused with small amount of human aPL IgG than pregnant wild-type mice [31]. On the other hand, when small amounts of human aPL IgG are administered to mice before implantation, placental histological analysis fails to show clear signs of inflammation [32]. Accordingly, abortive material or term placentae from APS women do not show any sign of acute local inflammatory events [2].

In vivo experimental models are also strongly suggestive for complement role in mediating aPL-induced pregnancy complications. Indeed, pregnant mice deficient in complement C3, C5, C5α receptor, or treated with an inhibitor of C3 convertase, do not experience aPL-induced foetal loss. Furthermore, the monoclonal anti-DI MBB2 induces foetal loss while the noncomplement fixing CH2-deleted variant does not [8]. In humans, a retrospective study finds complement deposition in placentae from aPL-positive women; a case study reported no complement deposition in foetuses miscarried by APS women, while a more recent prospective study on full-term placentas and abortive specimens shows only mild complement deposition without any relationship to pregnancy outcome or therapy [2].

Lastly, there is sound evidence for a direct effect of aPL on placentation. Polyclonal IgG from APS patients and human anti-β2GPI IgM monoclonals can react in vitro with β2GPI both at the foetal (trophoblast cells) and maternal (stromal decidual cells and human endometrial EC (HEEC)) sides of human placenta.

The direct interaction of aPL with the foetal side results in (1) inhibition of trophoblast differentiation, as shown by the reduced secretion of human chorionic gonadotropin, (2) impairment of the invasiveness of extravillous trophoblast cells, with a significant downregulation of matrix metalloproteinases, integrins, cadherins, and heparin-binding epidermal growth factor, (3) trophoblast injury and apoptosis, and (4) extrusion of necrotic trophoblast debris able
to activate the maternal endothelium. Upon interaction with the maternal side of the placenta, aPL are able to (1) induce a proinflammatory phenotype in stromal decidual cells and (2) block endometrial angiogenesis, by inhibiting HEEC angiogenic differentiation and production of proangiogenic factors [33].

3.5 RECEPTORS FOR β2GPI/ANTI-β2GPI ANTIBODIES

aPL-induced effects are mainly mediated by the reactivity of autoantibodies with β2GPI expressed on cell membrane. β2GPI adhesion to EC is mediated by several different receptors. AnnexinA2, a tPA and plasminogen receptor, directly binds β2GPI on EC and monocytes/macrophages. A coreceptor is required to trigger the signalling cascade because AnnexinA2 lacks an intracytoplasmatic tail. TLR2 and TLR4, heparan-sulphate and Apolipoprotein E Receptor 2′ (ApoER2′) have been shown to bind β2GPI on the endothelial surface [2,21]. In particular, TLR4 is the key player in driving endothelial perturbation: tlr4- but not AnnexinA2-silencing prevents the upregulation of adhesion molecules [34]. TLR4 is part of a multiprotein complex, which also includes AnnexinA2, calreticulin, and nucleolin, involved in aPL-induced EC activation [35].

On monocytes, aPL interact with β2GPI, AnnexinA2, and TLR4 within lipid rafts; however, β2GPI interaction with LPS accounts for apparent TLR4 activation by aPL. LPS/β2GPI complexes mediate β2GPI binding to cell membrane; both LPS and TLR4 are required for β2GPI to bind and activate macrophages, and treatment with LPS-inactivator polymyxin abolishes β2GPI binding to macrophages [36]. LPS/β2GPI complexes do not display the same effect on the endothelium. Indeed, low LPS contamination does not affect β2GPI/TLR4 interaction and EC activation; at high EC-activating concentrations, LPS increases β2GPI binding, likely through TLR4 upregulation. Cosilencing for AnnexinA2 and TLR4 does not completely inhibit anti-β2GPI antibody binding, a finding consistent with potential additional surface β2GPI receptors [34]. TLR1, TLR2, and TLR6 have indeed been appointed as candidate β2GPI coreceptors. TLR2 contributes to mediating intracellular aPL signalling in EC; to note, TLR2 is expressed by EC only upon cell activation while TLR4 is constitutively expressed. Other authors provide indirect evidence of TLR2 involvement in mediating aPL-induced monocyte activation [2,37]. TLR1, TLR2, and TLR6 colocalize with aPL IgG; antibodies blocking TLR1, TLR2, and TLR6 decrease aPL-mediated upregulation of TNF and TF in human monocytes [38].

AnnexinA2, TLR4, and ApoER2’ have also been investigated in vivo. Animals deficient in any of these molecules are only partially protected against aPL thrombogenic effects, suggesting redundancy in the signalling cascade [2]. The role of TLR2 and TLR4 was confirmed in a recent ex vivo study: peripheral blood mononuclear cells from APS patients display an increased mRNA expression of these innate immunity receptors and a markedly raised phosphorylation level of IRAK-1, a major mediator in the TLR transduction pathway [38].
To date, two cell membrane receptors appear to mediate aPL interaction with platelets. ApoER2′, a member of the low-density lipoprotein (LDL) receptor family, recognizes a positively charged patch of lysine residues in β2GPI-DV via its LDL-binding DI. An inhibitor of LDL receptors also blocks the platelet activation and thromboxane synthesis induced by aPL. Lastly, β2GPI binds directly to glycoprotein (GP) Iba, a subunit of GPIb-IX-V platelet receptor. The role of GP in APS pathogenesis is supported by in vivo findings: thrombus formation is not affected by aPL infusion in GPIIb/IIIa-deficient mice and pretreatment with a monoclonal anti-GPIIb/IIIa antibody inhibits aPL-mediated reduced thrombus formation [21].

3.6 INTRACELLULAR PATHWAYS

Upon the engagement of β2GPI receptors on target cells, aPL lead to the recruitment of nuclear factor κB (NFκB) and p38 mitogen-activated protein kinase (MAPK) in both EC and monocytes [2]. aPL engagement of NFκB occurs via a clathrin-dependent endocytic pathway, a mechanism requiring CD14 (TLR4 coreceptor) and AnnexinA2 [39].

The phosphatidylinositol 3-kinase (PI3K)-AKT pathway is an additional signalling cascade used by aPL. It culminates in the recruitment of mammalian target of rapamycin (mTOR), a kinase-modulating cellular growth, proliferation, and apoptosis. In human microvascular EC, stimulation with aPL IgG results in PI3K-mediated activation of two components of the mTOR pathway, S6 ribosomal protein (S6RP), and AKT [40].

Lastly, human monoclonal aPL and IgG fractions of APS patients induce transcription of NLRP3 and caspase-1 via the activation of endosomal NADPH-oxidase-2 (NOX2), resulting in inflammasome activation. Accordingly, mononuclear cells from APS patients show an increased expression of caspase-1 and NLRP3, with a threefold increased serum concentration of IL-1β [41].

The heterogeneity in the clinical spectrum of APS suggests that IgG from thrombotic versus obstetric patients might elicit different biological effects. In vitro support for this intriguing theory was first raised using monocytes: IgG from thrombotic APS patients, but not IgG from patients with obstetric APS, asymptomatic aPL carriers or healthy controls, cause NFκB and p38MAPK phosphorylation and TF upregulation [42]. The differential effects of IgG have been recently described using first-trimester trophoblast cells: aPL from women with pure obstetric APS, differently from those purified from thrombotic patients, which significantly reduce trophoblast invasion via TLR4 [43].

3.7 TWO-HIT HYPOTHESIS

Thrombotic events occur occasionally in patients with aPL, despite the persistent presence of autoantibodies. To tentatively explain this apparent paradox, a two-hit hypothesis has been formulated. In this, aPL, which act as the first
hit, might induce a thrombophilic condition that is not sufficient to trigger a clinically evident thrombosis. A second hit (namely, an additional thrombophilic condition) is required for clotting to take place [2]. Support for this hypothesis comes from animal models. aPL exert their pathogenic prothrombotic potential exclusively in animals already primed with LPS or mechanical, chemical, or photochemical trauma [2,19]. As infections frequently precede aPL-associated events, it has been suggested that infectious processes could be the second hit in humans [44]. This hypothesis fits well with the potential involvement of TLR2 and TLR4 in EC and monocyte activation by β2GPI-dependent aPL: β2GPI amount on resting endothelium is not enough to allow a sufficient aPL binding in order to trigger clotting. LPS may increase β2GPI vascular distribution by upregulating TLR2 and TLR4, thus overcoming the threshold for thrombosis [34]. In line with this hypothesis, LPS priming leads to the upregulation of β2GPI expression in murine tissues [45].

It is conceivable that the gut’s resident microbiota could affect LPS uptake, since it is the main source of LPS in healthy individuals. Gut microbiota is indeed increasingly recognized as a major player in the development of autoimmunity; commensal bacteria might contribute to APS pathogenesis inducing autoreactive CD4+ T-cells and anti-β2GPI antibody production via molecular mimicry mechanisms or favouring conformational changes in β2GPI. Consistent evidence comes from in vivo models: depletion of gut microbiome with broad-spectrum antibiotics in APS-prone animals markedly prevents thrombotic events, increases survival, and reduces anti-β2GPI IgG titres [46].

The two-hit hypothesis does not apply to obstetric APS: aPL IgG induces foetal loss in naive pregnant mice without requiring a second hit. β2GPI is largely expressed in placental tissues even in physiological conditions and binding of labelled exogenous β2GPI infused into naive pregnant mice to trophoblast and EC has been documented in vivo [45]. The high expression of β2GPI at the placental level together with pregnancy hormonal and blood-flow modifications might be sufficient to favour autoantibody pathogenic activity [4].

3.8 GENETICS AND EPGENETICS

The relevance of a genetic background for APS was first postulated in 1966, when Harvey described a family whose members, some with previous thrombosis, tested falsely positive for syphilis. When aPL positivity rates are evaluated in family members of APS patients, relatives of probands are more likely to carry aPL.

A segregation study on seven families with at least two members with APS (diagnosed according to a semi-quantitative scoring index different from international criteria) rejects an environmental and autosomal recessive hypothesis, suggesting an autosomal dominant model of disease inheritance. Since then, many genetic studies have focused on major histocompatibility complex genes that identify a strong association of APS with human leucocyte antigen (HLA)
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genes. DRB1*04, DR7, DQB1*0301/4, DQB1*0604/5/6/7/8/9, DQA1*0301/2 are described with increased frequency in patients with primary APS; anti-β2GPI antibody positivity is strongly associated with DRB1*1302 and DQB1*0604/0605 haplotypes in African-American and white British patients with primary APS, while in Caucasians and Mexican-Americans, DQB1*0302 strongly correlates with anti-β2GPI antibodies. Associations with non-HLA genes have also been investigated, with particular attention paid to single-nucleotide polymorphisms (SNP) in β2GPI. However, studies on Cys/Gly, Trp/Ser, and Val/Leu SNP provide conflicting results. A recent meta-analysis, specifically focusing on Val247Leu polymorphism, concludes that APS patients have a significantly higher prevalence of Val/Val genotype compared with controls; in particular, APS patients carrying anti-β2GPI antibodies have a higher prevalence, while no significant association emerges for arterial or venous thrombosis [47]. Weak associations have been observed with several SNP of immunoglobulin receptor FcγRIIA; a proinflammatory genotype, defined by SNP in the genes for IL1β, TNFα, TGFβ, IL6, and TLR4, that has been identified in APS patients within a single family. STAT4 and BLK, both associated with an increased susceptibility to lupus, exhibit a strong genetic association with APS, while a weak association for IRF5 and no association with BANK1 are observed.

Many studies report increased positivity rates of aPL in family members of lupus patients. This finding is also confirmed by a genome-wide linkage analyses on 1506 individuals: IgM, but not IgG aCL, exhibit a strong familial aggregation in these lupus pedigrees. Inherited prothrombotic factors may also modulate the thrombotic risk of aPL-positive subjects. FV Leiden is associated with increased thrombotic risk, while gain-of-function mutations in PT and loss-of-function mutations in AT, pC, and pS are linked to venous thrombotic events [48]. Epigenetics may also contribute to APS aetiopathogenesis, lowering the threshold for coagulation-cascade activation. An epigenetic mechanism that potentially plays a role in APS involves miR-19b and miR-20a, both down-regulating TF on monocytes; in particular, miR-20a might exert a direct regulating effect as it binds TF mRNA. In monocytes from APS patients, miR-19b and miR-20a are decreased, inversely correlating with TF expression on the cell membrane. However, the comparable expression of miR-19b and miR-20a in monocytes from aPL-negative lupus patients suggests the phenomenon is not APS-specific [49].

3.9 CONCLUSIONS

Research in APS has recently focused on the identification of novel mediators involved in the pathogenesis of the syndrome, also aiming at characterizing the diagnostic and prognostic value of each autoantibody subset. Several tools have been proposed to better discriminate between pathogenic versus nonpathogenic antibodies, such as the domain specificity of anti-β2GPI antibodies. Several
other antibody features are under evaluation; for example, the Fc glycosylation rate of anti-β2GPI antibodies might limit autoantibody pathogenicity [50]. Additional risk factors, such as ABO blood types, have been proposed as determinant of the hazard of each patient to develop clinical events [51]. Hopefully the next few years will yield the progressive unravelling of aPL-mediated pathogenic mechanisms and translate into a more accurate estimate of the thrombotic and obstetric risk, possibly leading to a treatment strategy tailored upon the characteristic profile of each patient.

REFERENCES


