Origin and pathogenesis of antiphospholipid antibodies

Abstract

Antiphospholipid antibodies (aPL) are a heterogeneous group of antibodies that are detected in the serum of patients with a variety of conditions, including autoimmune (systemic lupus erythematosus), infectious (syphilis, AIDS) and lymphoproliferative disorders (paraproteinemia, myeloma, lymphocytic leukemias). Thrombosis, thrombocytopenia, recurrent fetal loss and other clinical complications are currently associated with a subgroup of aPL designating the antiphospholipid syndrome. In contrast, aPL from patients with infectious disorders are not associated with any clinical manifestation. These findings led to increased interest in the origin and pathogenesis of aPL. Here we present the clinical features of the antiphospholipid syndrome and review the origin of aPL, the characteristics of experimentally induced aPL and their historical background. Within this context, we discuss the most probable pathogenic mechanisms induced by these antibodies.

Background

Antiphospholipid antibodies (aPL) occur in approximately 30% of patients with systemic lupus erythematosus (SLE) and in some patients with no defined connective tissue disease (1). Antiphospholipid antibodies are strongly associated with clinical complications such as arterial and venous thrombosis (2,3), thrombocytopenia (1,4) and recurrent spontaneous abortions (5,6). The presence of aPL in patient sera in association with these complications has been denoted since 1986 as the antiphospholipid syndrome (APS) (7). Other clinical features, such as stroke, pulmonary emboli, livedo reticularis, myocardial infarction and cardiac valve lesions, and Coomb’s-positive hemolytic anemia, were later included in the APS syndrome (8-11). APS is now defined as the presence of any of the above complications in association with aPL detected by either ELISA or lupus anticoagulant tests (8,11). Cardiolipin, a component of the inner mitochondrial membrane, is the common phospholipid used to detect aPL, also known as anticardiolipin antibodies (aCL) (1). APS may occur in the setting of SLE or other collagen vascular diseases (namely secondary APS) or in the absence of these disorders, being commonly known as primary APS (or PAPS) (10-12).

The report of APS and its clinical complications has led to increased interest in the origin and properties of aPL. Here we present the clinical manifestations of APS and review the origin of aPL based on experimentally
established aPL characteristics. Within this context, we discuss the most probable pathogenic consequences induced by these antibodies.

**Antiphospholipid antibodies and clinical associations**

**Thrombosis**

The most evident clinical manifestations of APS are thrombosis or thromboembolic vascular occlusions. Veins and arteries of all sizes and in any location may be involved. Thrombosis is usually recurrent and may be confined to a single location; however, some patients have thrombosis in both the arterial and venous circulation. Deep vein thromboses in the lower extremities are very common and may be complicated by pulmonary thromboembolism (13-15). Venous thrombosis may manifest as Budd-Chiari syndrome (16), hepatomegaly, pancreatitis, nephrotic syndrome (secondary to renal vein thrombosis (17)), Addison’s disease (18), pulmonary embolism, pulmonary hypertension, or retinal vein thrombosis (19).

**Thrombocytopenia and hemolytic anemia**

Thrombocytopenia in SLE is strongly associated with aPL (1,20-23). The IgG isotype of aPL has been detected in 72% of SLE patients with thrombocytopenia. Interestingly, 31% of 96 patients with chronic idiopathic autoimmune thrombocytopenia presented higher levels of aCL (21). In APS patients, thrombocytopenia is usually mild (>50,000 platelets/mm³) and may be associated with Coomb’s positive hemolytic anemia (Evans’ syndrome) (21-22).

**Cardiac complications**

APS patients may present ischemia or infarction of the left or occasionally right ventricle, secondary to coronary artery thrombosis (24). Hemodynamically significant valve lesions, vegetations, or pseudotumors (thought to result from endocardial thrombosis) have also been reported (25).

**Recurrent spontaneous abortion**

Recurrent spontaneous abortion is a major and frequent complication of APS (26). Women with SLE and aPL have a rate of fetal death up to six times higher when compared with healthy women (27,28). Moreover, among women with high levels of aPL, a history of prior fetal losses appears to compound the risk for further fetal demise. Increased awareness of this syndrome prompted a number of studies evaluating aPL in women with a history of recurrent fetal demise but with no known connective tissue disease (29,30). aPL levels were elevated in 13% to 42% of women with recurrent (usually three or more) miscarriages. Fetal death during the second or third trimester is more specific for aPL-associated fetal loss, but first-trimester spontaneous abortion is not uncommon. It was reported that 8% of women with first-trimester recurrent spontaneous abortions were aCL-positive (5,6). Placental insufficiency due to thrombosis is believed to cause fetal growth retardation, fetal demise, and preterm labor. There have been reports of thrombosis occurring in neonates of mothers with APS (31,32). However, the risk of these children developing APS is very small, and the major long-term complications appear to be secondary to low birth weight (33,34).

**Neurological complications**

Arterial thrombosis in patients with aPL often involves the cerebrovascular circulation and is expressed as stroke or transient ischemic attacks. Recurrent or chronic cerebral ischemia may manifest as intellectual/cognitive deterioration and dementia (35). Patients are often young, with the mean age
of patients with aPL and cerebrovascular ischemia being under 50 years (13). Thromboses of both small and large arteries as well as veins have been reported. The limited cerebrovascular histopathological data in both primary and secondary APS suggest a disturbance of microvascular coagulation with deposition of fibrin thrombi in small to medium-sized vessels (36), and only rarely has true cerebral vasculitis been documented (37). In some patients with aPL, cardiac emboli are a possible cause of cerebrovascular disease, perhaps contributing to large vessel thrombosis (25,35,36). The risk of recurrent stroke or transient ischemic attack is markedly increased in patients with aPL who have already had their first stroke. In a retrospective study of stroke in young patients, aPL positivity was associated with an eight-fold higher risk of recurrence (38). Multi-infarct dementia has been reported in APS patients (39), and the triad of recurrent strokes, livedo reticularis, and dementia (Sneddon’s syndrome) has been associated with aPL (40). Ocular ischemia in APS encompasses ischemic optic neuropathy, retinal artery occlusion, combined retinal artery and vein occlusion and amaurosis fugax (41).

In 1985, a woman with transverse myelitis, aPL, and a lupus-like disorder was reported (42). Transverse myelitis is an uncommon manifestation of SLE, occurring in approximately 1% of patients. Twelve such SLE patients had aPL, and six had thrombocytopenia, thrombosis, livedo reticularis, leg ulcers, and other clinical findings of APS. The pathophysiology of spinal cord damage in these patients is unknown, but ischemia or direct interaction of aPL with CNS phospholipids has been suggested (43). Several studies suggest an association between aPL and Guillain-Barré syndrome, seizures, chorea (44) and migraine headaches (45).

**Cutaneous lesions**

Livedo reticularis and other thrombotic skin lesions are recognized features of APS (46). Other cutaneous manifestations of APS include pyoderma-like leg ulcers, digital gangrene, and widespread cutaneous necrosis (47).

**Renal involvement**

In addition to renal vein thrombosis, accompanied by massive proteinuria, small-vessel non-vasculitic thrombi (thrombotic microangiopathy) may also occur in the kidneys of some APS patients (18).

**Antiphospholipid detection**

In 1906, Wassermann (48) described aPL as a serological marker for syphilis. In 1941, Pangborn (49) isolated and identified the antigenic component from bovine heart extracts as cardiolipin (diphosphatidylglycerol). Cardiolipin is a phospholipid unique to biomembranes which have coupled phosphorylation and electron-transport, i.e., mitochondria, chloroplasts, chromatophores, and bacterial plasma membranes (50). Cardiolipin, together with lecithin and cholesterol, is used as the antigen in a serodiagnostic flocculation test for syphilis referred to as the VDRL (Venereal Disease Research Laboratory) test. When specific tests for treponemal antibodies, such as the *Treponema pallidum* immobilization test, were developed (51), it became clear that not all individuals with a positive Wassermann or VDRL reaction had syphilis. The term “biological false-positive serological test for syphilis” (BFP-STS) was then introduced. Moore and Mohr (52) identified two distinct groups of patients: one with transient BFP-STS reactions, usually associated with viral or other infections, and the other with BFP-STS results persisting for a period of six months or more (termed chronic BFP-STS). The latter had a high prevalence of autoimmune disorders such as SLE, Sjögren’s syndrome, autoimmune hemolytic anemia, Hashimoto’s thyroiditis,
and rheumatoid arthritis (53).

A report of two patients with hemorrhagic disorders and prolonged prothrombin times as well as BFP-STS prompted speculation about the relationship between BFP-STS and a circulating lupus anticoagulant (LA), identified in the gamma globulin fraction of patient sera (54). Though LA was originally associated with bleeding, later work showed that this association is rare and that, paradoxically, LA is more often associated with thrombosis (2,3). LA competes for phospholipids with other coagulation factors. Adequate testing for LA requires freshly prepared platelet-depleted plasma and therefore cannot be performed on stored serum or inadequately prepared plasma. Dissatisfaction with the lack of sensitivity of the VDRL test and difficulties in measuring LA increased together with clinical awareness of the association between antiphospholipid antibodies and thrombosis, thrombocytopenia, and recurrent spontaneous abortions. These facts led investigators to devise a simple and reliable technique for detecting and characterizing aPL. In 1983, Harris et al. (1) designed a radioimmunoassay using cardiolipin as antigen, later converted to an enzyme-linked immunoassay (ELISA) (55). Standard calibrators (samples with defined international units) for IgG, IgM, and IgA aPL have been established, allowing interlaboratory comparison of results (55). Although aPL and LA can be separated by affinity chromatography or cardiolipin liposome adsorption (56), whether aPL and LA share a similar epitope or are completely different antibodies is not known.

**Characteristics of antiphospholipid antibodies**

Antiphospholipid antibodies are usually autoimmune or infection-induced; less frequently aPL may be drug-induced (57,58) or occur in patients with lymphoproliferative disorders. The association of non-autoimmune aPL with clinical complications remains controversial. Research has been focused on both types of aPL (autoimmune and infectious) in an attempt to gain a better understanding of their pathogenicity. Differences in aPL isotype (59), IgG subclass (60), light chain distribution (61), antibody avidity (60,61), phospholipid specificity (62), and cofactor requirement (63-65) for autoimmune and infectious aPL have been identified and are detailed below.

The clinical complications are strongly associated with aPL of the IgG isotype (59,66-68). However, in the absence of IgG aPL, IgA and IgM aPL also appear to be associated with clinical complications. In a retrospective study of 40 APS patients, 36 (90%) were found to have IgG aPL (alone or with other isotypes), while the remaining four had complications associated with IgA and IgM alone (59). Post-infectious aPL are usually low-titer (when detected by ELISA), with the exception of syphilis, when high-titer IgG aPL may occur (61). The IgG subclass of autoimmune aPL may reflect the nature of the antigen that triggers pathogenicity. Among autoimmune aPL, IgG2 is predominant (61,69), while IgG1 and IgG3 are the most common subtypes of anti-DNA, anti-nuclear protein antibodies, and of other autoantibodies (58,70,71). Interestingly, the predominant IgG subclasses for syphilitic aPL are IgG1 and IgG3. The light chain type of autoimmune aPL is predominantly lambda (61,69), while the normal light chain distribution is approximately two-thirds kappa and one-third lambda. The primary light chain of other autoantibodies and syphilitic aPL is kappa (61).

Avidity of aPL for phospholipids, when compared with other antibodies to protein antigens, is generally low. As an illustration, aPL-phospholipid complexes dissociate in the presence of 1 M salt (56,72). aPL purification is still not a standard procedure, partly because the principal epitope recognized by aPL remains uncertain. Therefore, avidity
measurements are usually presented for a specific aPL isotype. Autoimmune aPL appear to have higher avidity for negatively charged phospholipids than do syphilitic aPL when ELISA-based methods are used (61).

While designing the aPL ELISA, investigators noticed that the use of whole bovine serum as test sample diluent reduced background values and enhanced antibody binding to cardiolipin-coated plates. This suggested the presence of a cofactor for aPL in bovine serum that was later identified as β2-glycoprotein I (β2GPI), also known as apolipoprotein H (64,65). Human β2GPI is a glycosylated, proline-rich polypeptide composed of 326 amino acids with multiple disulfide bridges. These bridges divide the protein into five short consensus repeats known as ‘sushi’ domains (73), the last of which is thought to be most important in binding negatively charged macromolecules such as heparin, DNA, and negatively charged phospholipids (64,65). Although the physiologic role of β2GPI is unclear, β2GPI may function as a natural regulator of coagulation by its anticoagulant properties, including inhibition of the contact system of blood coagulation (74) and inhibition of the intrinsic pathway and adenosine diphosphate (ADP)-induced platelet aggregation (74-76). Autoimmune and drug-induced IgG and IgM aPL require β2GPI for binding to phospholipids (PL). In contrast, the PL binding of aPL associated with syphilis, HIV, or other infection-induced antibodies is inhibited by β2GPI (63,77). This divergence in the phospholipid binding by autoimmune and infectious aPL supports the proposal of a different origin for these two groups of aPL (61,78).

Although the degree of enhancement by β2GPI may vary (68), autoimmune IgM aPL require lower levels of cofactor than do IgG aPL (79). These findings incited the ongoing controversy regarding the target epitopes recognized by autoimmune aPL. PL modified by β2GPI, PL complexed with β2GPI (78), and a cryptic epitope on β2GPI (or other native proteins) that is exposed when this cofactor binds to cardiolipin (80) are discussed as the target epitope for aPL. Regardless of the identity of the target epitope, the conventional aPL ELISA is the most reliable method of detecting autoimmune aPL for clinical diagnosis. This method uses anionic PL-coated plates and provides sufficient bovine β2GPI in the diluent (10% bovine serum). Furthermore, test sera at 1:50 dilution provide an additional 4 mg/ml of human β2GPI for the assay, enough cofactor for the PL-binding and aPL detection (68).

Although the ELISA test is widely used for investigating the phospholipid-binding properties of aPL, the form adopted by the phospholipids in the ELISA plates is unknown. Soluble microparticles or cell fragments are the most probable phospholipid target epitope for aPL circulating in vivo. For studies in solution, unilamellar liposomes, in which the phospholipids are organized in bilayers, have been adopted as a biomimetic model (81). Using electron paramagnetic resonance, it was demonstrated that purified IgG aPL from SLE or APS patients and from syphilis patients induce superficial membrane defects (in the polar head group region) in liposomes composed of different molar ratios of cardiolipin and phosphatidylcholine. Another distinct aPL property was reported with this technique: autoimmune aPL, but not infectious aPL, induce packing lipid defects in the core of the bilayer (82). β2GPI-free IgG aPL from autoimmune and infectious diseases, shown to bind to cardiolipin in the absence of β2GPI in a modified ELISA (83), induced the leakage of the internal contents of these liposomes, detected by carboxyfluorescein fluorescence (82,84,85). Purified native and recombinant human β2GPI itself induced the leakage of the liposomes in a temperature- and concentration-dependent manner (86). In this liposome model, β2GPI presented the cofactor effect inducing a synergistic increase in the leakage rate induced by autoimmune IgG.
aPL (84,86). β₂GPI requirement for the maximum leakage rate was inversely related to IgG aPL affinity for cardiolipin (86). As expected, β₂GPI inhibited the leakage from the liposomes induced by infectious IgG aPL (85). These findings suggest that the epitope recognized by autoimmune aPL can be the phospholipid itself or most likely the complex of β₂GPI-phospholipid. Besides, the inhibition data strongly indicate that β₂GPI is not part of the epitope recognized by aPL detected in syphilis patients. Altogether, these conclusions show that aPL from autoimmune and infectious diseases are originated by different induction mechanisms, a fact that may explain the divergence in the association of the clinical manifestations (specific for aPL from autoimmune disorders).

**Pathogenicity of antiphospholipid antibodies**

The close association of autoimmune aPL with clinical complications such as thrombosis and spontaneous abortion suggests a pathogenic role for these antibodies but does not prove that aPL cause these complications. More convincing evidence that aPL are not merely epiphenomena or surrogates for the true causes of thrombosis comes from experimental models of APS.

APS has been passively transferred by injection of pregnant BALB/c mice with purified IgG from patients with aPL-associated recurrent spontaneous abortions (87,88). The induction of aPL in mice and rabbits by immunization with foreign (human) β₂GPI has been reported (89,90). Induction of aPL in young (two to four months) PL/J mice by this method resulted in intrauterine fetal death as well as neurological complications resembling transverse myelopathy (91).

The mechanisms by which aPL may cause thrombosis are unclear, but several have been suggested, such as alteration of the eicosanoid balance (92), interaction of the aPL with the protein C-protein S pathway (93), inhibition of the anti-thrombin III activation (94), activation of platelets (95) and endothelial cells by aPL (96,97) and finally, the interference of aPL with the anticoagulant functions of natural inhibitors of coagulation such as β₂GPI and placental anticoagulant protein I (also known as annexin V) (98-100). These mechanisms have been elucidated recently (101).

**Origin of antiphospholipid antibodies**

It seems likely that autoimmune aPL are produced during antigen-driven immune responses in subjects with a particular genetic background. The precise nature of the inciting antigens and the major genes involved are unknown. Pure phospholipids are not immunogenic, and immunization of laboratory animals with phospholipid plus adjuvant does not induce aPL. Immunization of mice or rabbits with purified, lipid-free, heterologous β₂GPI, a phospholipid-binding protein, induces aPL with properties similar to autoimmune aPL (89,102). We believe that foreign β₂GPI binds PL in vivo, thus forming an immunogenic complex against which antibodies are produced. To test this hypothesis and to determine whether the presence of intact β₂GPI is required, we immunized mice with a synthetic pentadecapeptide that spans Gly 274-Cys 288 in the fifth domain of human β₂GPI and contains the putative PL binding site (103). Immunization with this peptide (which we have termed “GDKV” conjugated to carrier proteins (BSA or KLH), but not the peptide alone, induced aPL production in two normal (NIH/Swiss and PL/J) strains of mice (104). These studies suggest that the natural stimulus for autoimmune aPL may be a foreign protein (e.g., a viral or bacterial product) with PL-binding properties similar to those of β₂GPI or the GDKV-BSA conjugates.

Further studies are necessary to answer
the various remaining questions about aPL. The research about the origin of aPL is directed not only at the identification of the pathogenic mechanisms that lead to the clinical manifestations, but mainly at the development of better treatments for SLE and APS patients.

Acknowledgments

We are grateful to Dr. Hernan Chaimovich, PhD, for fruitful discussions and revisions.

References


27. Branch DW, Scott JR, Kochenour NK & Hershgold E (1985). Obstetric complications associated with the lupus antico-
64. Netsuma E, Igarashi Y, Fujimoto M,


