ANTIPHOSPHOLIPID ANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS AND THE ANTIPHOSPHOLIPID SYNDROME

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1. ABSTRACT

The family of autoantibodies known as antiphospholipid antibodies (aPL) and the lupus anticoagulant (LA) are associated with a spectrum of clinical manifestations including life-threatening thrombosis. While our current knowledge of thrombosis is imperfect and the mere presence of aPL is imprecisely associated with clinical events, our knowledge in this area has greatly expanded in recent years. It is clear that high levels of IgG aPL are associated with an increased risk of thrombosis.

In 1990, investigators demonstrated that some aPL are directed against the beta$_2$-Glycoprotein I (beta$_2$-GPI) 50 kDa subunit and reported that these showed concordance with risk of clotting in certain groups of patients. Studies have also demonstrated that aPL reacted with antigens other than beta$_2$-GPI, namely prothrombin, annexin V, protein S, protein C and high molecular weight kininogen.

We review the clinical features of the antiphospholipid syndrome (APS), including vascular occlusion, pregnancy loss, thrombocytopenia and catastrophic APS. We also review the role of antibodies in the pathogenesis of APS as well as the spectrum of autoantibodies that have been found in APS.

2. INTRODUCTION

In 1952 Conley and Hartman recognized a phenomenon occurring in patients with systemic lupus erythematosus (SLE) that resulted in the prolongation of normal tests of coagulation, an apparent anticoagulant. In 1977, Feinstein and Rappaport called this factor the lupus anticoagulant (LA), and it was subsequently shown that these anticoagulants were associated with a biologically falsely positive test for syphilis and paradoxically predisposed patients to thrombosis (1). The first antiphospholipid antibody (aPL) to be recognized was the biologically false- positive test for syphilis. In the 1940s Mary Pangborn identified the antigenic component in the tissue extracts used in these tests derived from the beef's heart, as a novel anionic phospholipid, named cardiolipin (2,3). The syndrome associated with these antibodies, lupus anticoagulant and antiphospholipid antibodies, with these related clinical implications, are now well defined as the antiphospholipid syndrome APS, which has gained worldwide acceptance. The current thought is that biologically false positive test for syphilis is not considered a strong associate of APS. Synonyms for APS are lupus anticoagulant, Hughes' syndrome and anticardiolipin antibody syndrome.

The APS is a clinical syndrome defined by the occurrence of arterial and/or venous thrombotic events;

Table 1. Manifestations of APS
Major
- Arterial thrombotic events
- Venous thrombotic events
- Recurrent pregnancy loss
- Thrombocytopenia

Minor
- Livedo reticularis and leg ulcers not related to venous insufficiency
- Cerebral ischemia, dementia, migraine, chorea and seizures
- Hemolytic anemia

Any one of the first three major manifestations in the presence of positive test for aCL antibody or lupus anticoagulant is necessary to diagnose APS (12).

often multiple, recurrent fetal loss, and/or thrombocytopenia with positive results for lupus anticoagulant and/or antiphospholipid antibodies. The spectrum of the so called aPL antibodies include the anticardiolipin antibodies (aCL) measured by solid phase enzyme linked immunosorbent assay (ELISA) and lupus anticoagulant measured by the prolongation of phospholipid dependent coagulation time. These tests have been used to diagnose the APS worldwide.

The aPL is a misnomer because the antibodies are directed not only at the negatively charged phospholipid, but are primarily directed at protein-phospholipid complexes. Several investigators have shown that some of the aPL are directed at beta$_2$-glycoprotein I (beta$_2$-GPI) which appears to be a protein cofactor necessary for enhancing antigen binding for some of these phospholipid antibodies. Subsequent methods have been developed to detect antibodies against beta$_2$-GPI (anti-beta$_2$-GPI) (4,5,6,7,8,9,10,11).

The APS may occur alone, with no clinical or serologic evidence of another definable disease in which case it is known as primary APS. The occurrence of APS in the setting of another primary disease is called secondary APS. This occurs with diseases such as lupus, rheumatoid arthritis, systemic sclerosis, vasculitis, infection, malignancy and drug induced lupus-like syndromes.

3. CLINICAL FEATURES

APS is associated with major and minor manifestations. The 4 major manifestations are arterial thrombotic events, venous thrombotic events, recurrent pregnancy loss and thrombocytopenia. Minor manifestations continue to be described and have been reported in individual patients, case series or cross sectional studies and may be due to coincidence or chance. Over the past few years there has been increased awareness of the catastrophic APS (table 1).

3.1. Vascular Occlusion

In studies on the natural history of the aPL-positive patients, it has been shown that fewer than 50% of the aPL-positive SLE patients and 31% of all aPL-positive

patients ever experience thrombosis. Furthermore, approximately 76% of those patients who initially experience a venous thrombosis are at an increased risk to sustain another event, and 93% of those who have had an arterial event have another arterial event if recurrent thrombosis occurs (13). These thrombotic events can be severe and occur at unusual anatomic locations. Factors capable of triggering a clinical thrombosis in patients with APS include pregnancy, postpartum status, exogenous estrogens, elective surgery and infection. Patients with secondary APS appear to be at risk for recurrent thromboses during flares of their disease.

3.2. Pregnancy loss

Recurrent fetal loss is one of the most emotionally distressing aspects of the APS. It is believed to be a consequence of thrombosis of the placenta (multiple microthrombi can be visualized on the maternal side of the placenta) such that the placenta can sustain the pregnancy only for a limited period of time. Although loss of the pregnancy in patients with aPL typically occur after 10 weeks of gestation, earlier losses can occur. In patients with aPL, prior pregnancy losses predict future losses independent of aPL titer (14).

3.3. Thrombocytopenia

Several studies have shown a strong statistical correlation between presence of the aPL and thrombocytopenia (15). These antibodies may mediate platelet destruction by cross-reaction with phospholipid present in platelet membranes. Antiphospholipid antibody of immunoglobulin class G was elevated in 31(72%) of the 43 patients with thrombocytopenia (16).

3.4. Catastrophic APS

A small number of patients with APS develop an acute onset of vascular occlusions of medium and small sized arteries causing cerebro-vascular, cardiac, hepatic, adrenal, intestinal and renal infarction, and peripheral gangrene of medium and small sized arteries in multiple organs, and results in a life-threatening situation (17). The differential diagnosis in these patients is often difficult and lies between lupus vasculitis, thrombotic thrombocytopenic purpura (TTP), and disseminated intravascular coagulation (DIC). The fact that erythrocytes are not usually fragmented, fibrin split products are not strikingly elevated and absence of microangiopathic hemolytic anemia will assist in differentiation. Tissue biopsies and histopathology in such patients reveals non-inflammatory vascular occlusions.

3.5. Minor manifestations

Neurologic syndromes associated with APS include cerebral ischemia, ocular ischemia, dementia, migraine headaches, transverse myelopathy, Guillain Barre's syndrome and seizures. Cerebral ischemia is the most common neurologic symptom associated with aPL antibodies. The risk for recurrent stroke appears to be markedly increased in patients with aPL who have already suffered their first strokes. Regardless of age, patients with cerebral or ocular ischemia may have other risk factors for cerebrovascular disease. Neuro-imaging studies in patients

Table 2. Autoantibodies Associated with APS
  Anti-cardiolipin antibodies
   • Anti-beta$_2$-GPI antibodies
   • Anti-cardiolipin antibodies (directed to negatively charged phospholipids such as cardiolipin and phosphatidylserine)
  Lupus anticoagulants
   • Anti-beta$_2$-GPI antibodies
   • Anti-prothrombin antibodies
  Other autoantibodies
   • Anti-annexin V antibodies
   • Antibodies against high- or low-molecular kininogen
   • Antibodies against protein C or protein S

with neurologic manifestations of APS have revealed small foci of high signal intensity subcortical white matter lesions that are scattered throughout the brain (18).

Cutaneous manifestations include livedo reticularis, acrocyanosis, Raynaud's phenomenon and leg ulcers not related to venous insufficiency. Livedo reticularis may be a marker for the later development of CNS disease, renal disease and vasculitis (19).

Pulmonary manifestations include pulmonary embolism, pulmonary infarction and chronic thromboembolic pulmonary hypertension. Diffuse alveolar hemorrhage has been an occasionally reported manifestation of APS and this may result in life threatening symptoms (20). Hemolytic anemia also can occur in addition to thrombocytopenia.

Surprisingly, there was relative lack of association between the presence of aPL and clinical manifestation of thromboses when a group of patients with positive autoantibodies to U1-70 Kd were studied (21); the only clinical manifestation found to be associated with this group of patients is pulmonary hypertension (22).

4. PATHOGENESIS

APS is a complex autoimmune disorder in which various autoantibodies can be involved possibly leading to diverse serologic and clinical manifestations associated with autoimmune diseases. The mere presence of this heterogeneous group of antiphospholipid antibodies and/or lupus anticoagulants, in lupus patients and lupus-like syndromes neither predicts the likely incidence of life threatening thrombotic events nor indicate when such an event can occur. However, it is suggested that these autoantibodies play a pathogenic role leading to hypercoaguable states; clinically high levels of IgG isotype of aPL concur with increased risk of thrombosis (23). There are several important differences found in the antigenic specificity and clinical manifestations of these aPL found in patients with lupus versus aPL associated with infection. Infection-associated aPL are usually less cross reactive to negatively charged phospholipids but rather specific for cardiolipin, are often transient, low in titers by solid phase assay are of the IgM isotype (24) and importantly not dependent on beta$_2$-GPI for binding (25).

On the other hand, autoimmune associated aPL are persistent, often high titers of IgG isotype predominantly IgG2 and IgG4 subclasses and react with all negatively charged phospholipids (26,27). In 1990 several investigators showed that the anticardiolipin antibodies are directed to the fifth domain of the beta$_2$-GPI a 50-kDa protein. Methods were developed to detect antibodies against beta$_2$-GPI (anti-beta$_2$-GPI), and it has been shown that their presence correlated with increased possibility of thrombosis in patients with SLE and primary aPL syndrome (5,6,7,8,9). The study of anti-beta$_2$-GPI has allowed major advances in the field of APS, and has shed light into the pathogenesis to such an extent that the syndrome is now called as the "antiphospholipid co-factor syndrome" (10,11). Is anti-beta$_2$-GPI more important than aPL as risk factors for thrombosis and other clinical manifestations in SLE and lupus-like diseases? It is suggested that patients with both aPL and anti-beta$_2$-GPI are at a greater risk for thrombosis compared to patients that carry only one of these autoantibodies. Some authors have suggested lupus patients with the presence of anti-beta$_2$-GPI but without antibodies in the antiphospholipid assays may represent a variant of antiphospholipid co-factor syndrome (28).

Several studies now support that aPL react with antigens other than beta$_2$-GPI, namely prothrombin, annexin V, protein S, protein C and high molecular weight kininogen (table 2).

4.1. Antibodies against beta$_2$-Glycoprotein

The anti-beta$_2$-GPI can be classified into several groups. One group of antibodies are aCL dependent and directed against beta$_2$-GPI, derived from autoimmune disease patients where the binding of autoimmune cardiolipin antibodies is enhanced by the presence of anti-beta$_2$-GPI. A second group of anti-beta$_2$-GPI are made in response to infections and have little relationship to thrombosis.

Numerous putative mechanisms have been hypothesized to explain how anti-beta$_2$-GPI predispose to thrombosis and accelerated atherosclerosis. Despite great interest in this phospholipid protein binding antibody and its role as an antigenic co-factor in autoimmune response in APS, its physiological role is unclear. Beta$_2$-GPI (also known as apolipoprotein H) does interact with lipoproteins and its role in lipid metabolism may explain its role in atherosclerosis. The annexin V hypothesis of the antibody mediated aCL and anti-beta$_2$-GPI displacing the anticoagulant shield from the endothelial membranes and placental trophoblasts has been proposed, although hypothesis is still challenged.

It is now well appreciated that activated platelets play an important role in thrombosis. These are the major source of negatively charged phospholipids (phosphatidylserine and phosphtidylionositol, etc) that provide a catalytic surface for coagulation. In the coagulation cascade one of the most important steps is activation of factor X to activated factor X (Xa) the presence of additional phospholipid dependent coagulation

factors V, Ca^2 and prothrombin. Anti-beta$_2$-GPI inhibited the generation of coagulation factor Xa by activated platelets by directly interfering with this inhibition (29).

Anti-phospholipid antibodies may affect platelets by binding directly to the platelet wall and thereby accelerating platelet adhesion and aggregation. Ultimately, this may lead to thrombus formation. Secondly APS may have a thrombotic effect on the vascular endothelium by binding to the phospholipids in the endothelial cell wall and interfere with the normal production of prostacyclin (a naturally occurring vasodilator and a potent inhibitor of platelet aggregation). Additionally, there exists some evidence that anti-beta$_2$-GPI may damage the endothelial cell wall, thereby decreasing production of endothelial derived relaxing factor. This would render the vessel prone to vasospasm, presumably due to ischemic vessel damage and thrombosis. It is also hypothesized that anti-beta$_2$-GPI bind to the endothelial cell surface may induce expression of adhesion molecules such as I-CAM-I, V-CAM-I and E-selectin. Interleukin (IL)-6 and 6-keto-PGF alpha secretion enhance monocyte adhesion to the endothelial cells (29,30). The precise nature of the beta$_2$-GPI receptors, and the mechanism by which antibody binding to beta$_2$-GPI occurs are not known, however.

It has been recently shown that oxidized LDL (oxLDL) and not native LDL aggravate manifestations of APS. Interaction between oxidized plasma lipoproteins (oxLDL, oxVLDL, oxHDL) and beta$_2$-GPI and anti-beta$_2$-GPI has been shown. Anti-beta$_2$-GPI bound to beta$_2$-GPI are complexed with oxidized forms of the lipoprotein. The binding of oxLDL complex to macrophages was inhibited in the presence of beta$_2$-GPI, whereas it was enhanced in the presence of beta$_2$-GPI and anti-beta$_2$-GPI. These findings suggest that oxLDL is targeted not only by antibodies to negatively charged phospholipids but by also anti-beta$_2$-GPI via beta$_2$-GPI adhesion. Thus, aPL may be involved not only in the lipoprotein metabolism but also in atherogenesis (31,32).

4.2. Antibodies against prothrombin
Anti-prothrombin antibodies were present in the sera of 70% of patients with lupus anticoagulant in the setting of infection or malignancy (33,34). These antibodies were seen in associated with SLE or primary APS and only 20% with lupus coagulants associated with patients both with and without hypoprothrombinemia.

4.3. Antibodies against annexin V
Anti-annexin V antibodies are found in the vascular endothelium and placenta among many other tissues (35). Annexin V is capable of displacing coagulation factors from phospholipid surfaces hence it is a potent anticoagulant. In patients with APS it is hypothesized that the annexin V levels are reduced at sites where circulating blood contact the cells lining the vasculature. In the placental villi this leads to increased risk of thrombosis (36,37).

4.4 Antibodies against kininogens
Kininogen is required for the binding of prekallikrein and factor XI. It is both a substrate and a co-factor in the contact phase of coagulation. High and/or low molecular weight kininogens coupled aPL (namely phosphatidylethanolamine found in patients with SLE) increase the risk of recurrent spontaneous abortions (37).

4.5. Antibodies against protein C and protein S
Protein C functions as an anticoagulant that inactivates factor Va and VIIa limiting thrombin formation. Decreased levels of this factor predispose to increased thrombosis. It has been demonstrated that antibodies inhibiting factor Va degradation are directed against phospholipid bound protein C or protein S.

5. CONCLUSION

Currently available clinical and serologic evidence strongly suggests that aPL, aCL, and other antibodies directed against clotting factors are associated with a definable clinical syndrome, APS. The precise pathogenic mechanism of recurrent thrombosis are currently being defined. Anti-beta$_2$-GPI autoantibodies and anti-prothrombin antibodies play an important role in the pathogenesis of thrombosis and recurrent fetal loss. Further research is warranted to elucidate these mechanisms of both thrombosis and fetal loss associated with autoantibodies. Improved standardized specific laboratory testing allows for better detection of these autoantibodies and for the management of APS.

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