5. PATHOGENESIS OF ANTI-PHOSPHOLIPID SYNDROME

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5.1 Introduction

The antiphospholipid syndrome (APS) is an autoantibody-mediated autoimmune disorder in which thrombosis occur in patients having laboratory evidence for so-called antiphospholipid antibodies (APL). The association of thrombosis, recurrent foetal losses and thrombocytopenia with the lupus anticoagulant (LA) phenomenon was observed forty years ago (1,2,3,4,5,6), but it was not until eighties that Hughes referred to the possible existence of a syndrome (7,8). The name of anticardiolipin syndrome was soon replaced by APS (9) known also as Hughes’s syndrome. A wide spectrum of clinical and basic science disciplines has made important contributions to the knowledge of APS: immunology, rheumatology, obstetrics, neurology, haematology, molecular biology, lipid and protein chemistry etc.

5.2 Clinical and laboratory criteria

Criteria for the classification of the APS raised after many international multidisciplinary symposia and workshops and included clinical and laboratory findings (10). Definite APS is considered to be present if at least one of the clinical and one of the laboratory criteria are met (Table 1).

| Table 1. Criteria for the classification of the APS, (According to Wilson et al. Arthritis Rheum. 1999;42:1309-1311) |
|--------------------------------------------------|
| **Clinical criteria**                             |
| one or more clinical episodes of arterial, venous, or small vessel thrombosis in any tissue or organ, one or more unexplained deaths beyond the 10th week of gestation, or three or more unexplained consecutive spontaneous abortions before the 10th week of gestation or premature births before 34th week of gestation because of a severe preeclampsia or eclampsia or placental insufficiency. |
| **Laboratory criteria**                           |
| Anticardiolipin antibodies of IgG and/or IgM type in blood, present in medium or high titer, on 2 or more occasions, at least 6 weeks apart, measured by a standardized enzyme-linked immunosorbent assay for beta2-glycoprotein I dependent anticardiolipin antibodies. |

Many clinical features were reported to be found in APS (The reader is referred to ref 11) but they are not all included in the criteria: thrombocytopenia, haemolytic anaemia, cardiac valve disease, transient cerebral ischaemia, stroke, transverse myelopathy, myelitis, chorea, livedo reticularis, migraine, cognitive dysfunctions and others. For them additional studies are encouraged.

It should be born in mind that APL are heterogeneous group of antibodies (Readers are referred to ref. 12 for historical background). APL were first identified as a group of antibodies recognizing epitopes on anionic and neutral phospholipids (cardiolipin, phosphatidyserine, phosphatidylethanolamine). In enzyme-linked immunosorbent assay (ELISA) the first reagent is cardiolipin, and the APS thus obtained are called anticardiolipin antibodies. In fact, the real antigens are cardiolipin-bound serum proteins, whereas only a minor part of antibodies bind directly to phospholipids. The later are more common in infectious diseases and are mainly not related to the development of thrombosis. In patients with APS, the antibodies are primarily directed against phospholipid-binding plasma proteins. Among these, K2-glycoprotein I, prothrombin, annexins, protein C, protein S, high molecular weight kininogen have been described (13,14,15,16,17,18). Some of them have been proved to possess lupus anticoagulant activity.

5.3 Proposed pathogenic mechanisms

If we consider the wide spectrum of clinical features and a great heterogeneity of APL, the single pathogenic mechanism is very unlikely. Even thrombotic events in patients with APL segregate into venous and arterial episode, and recurrence is usually on the same part of the vascular system (venous or arterial). This implicates that thrombotic mechanisms are heterogeneous. The demonstration of APL pathogenicity is given by the possibility of inducing APS in the mouse experimentally by passive APL transfer (19), but no conclusive and direct evidence yet exists that APL per se are pathogenic or are direct mediators in the development of thrombotic events in humans.

Several mechanisms were suggested for developing clinical manifestations related to the APS. The proposed
pathophysiological mechanisms for thrombosis may be grouped into

a) inhibition of anticoagulant reactions and

b) alteration of cell expression and secretion.

The protein C pathway is one of the important endogenous antithrombotic mechanisms. Protein C is activated by the thrombomodulin-thrombin complex and activated protein C inhibits procoagulant factors Va and VIIa. Protein S supports the anticoagulant potential of activated protein C. APL can interfere with the protein C pathway in different ways, via protein C activators (20, 21), protein C inhibitors (22) or directly (23, 24, 25), resulting in an acquired resistance to activated protein C. Non-inactivated factors Va and VIIa promote coagulation and increase susceptibility to venous thrombosis (26). APL-induced protein C dysfunction is mediated by ß2GPI, but autoantibodies against thrombomodulin, prothrombin, protein C and protein S have been reported in some APS patients as well (16, 17, 27, 28, 29, 30).

Antithrombin III is an important inhibitor of coagulation factors Va and thrombin. APL may also affect its activity through cross-reactivity with highly polyanionic heparinoid molecules (e.g. heparan sulphate proteoglycans with thrombomodulatory effect) and promote hypercoagulability state (31, 32).

On the other side, APL also perturbs fibrinolysis. Abolished regulatory mechanism of protein C prolongs biological half-life of tissue plasminogen activator/inhibitor and may contribute to the development of arterial or venous thrombosis due to reduction in fibrinolytic activity. In addition, impaired fibrinolysis has been found in association with antibodies directed against endothelial cell and manifests endothelial cell dysfunction (33).

The endothelial cells have an important role in the regulation of spontaneous activation of the coagulation system. It has been suggested that APL induce a pro-coagulant / pro-thrombotic phenotype of endothelial cells by different mechanisms. APL, bound to endothelial cells, have been shown to induce up-regulation and increase synthesis of adhesion molecules E-selectin, intracellular cell adhesion molecules 1 (ICAM1), vascular adhesion molecules 1 (VCAM1) and the secretion of pro-inflammatory cytokines (IL-1ß, IL6) (34, 35, 36).

It looks as though APL are associated with up-regulation tissue factor mRNA (37, 38) which is the physiological initiator of normal coagulation. Normally it is not expressed in a functionally active form on cells in contact with flowing blood. Considering that the physiological activation of the tissue factor pathway must be very fast, however, it seems unlikely that transcription alone regulates its activity. It is thought that tissue factor may be present on a cell surface but in encrypted form. Some data suggest that tissue factor dimer is inactive (encrypted), while monomer is active (39). The exact mechanism of APL involvement in monomerisation or other type of tissue factor activation on vascular endothelial cells and monocytes is not clear yet (40, 41).

Annexins are a family of calcium-dependent phospholipids binding proteins with high affinity for anionic phospholipids and potential anticoagulant activity in vitro. It was hypothesized that thrombosis and fetal loss in APS are due to APL-mediated displacement of an antithrombotic (anticoagulant) annexin V shield on vascular endothelium and placental throphoblasts (43, 44, 45). This is controversial area with inconsistent results (46) and some patients were reported with neurological disorders and the presence of anti-annexin V antibodies, not fulfilling the laboratory criteria for APS (47).

APL together with ß2GPI cause in vitro more pronounced budding and vesiculation of phospholipid vesicles, resulting in increased number of micro-particles (48). Vesiculation of endothelial cells in vivo (49) or platelets together with membrane budding increase the expression of proadhesive and pro-coagulant surface in APS. It has been demonstrated that APL can stimulate platelet aggregation by direct Fab fragment binding or by complex binding to platelets FcRII receptor. Through these interactions a vicious circle of cellular activation may be created, ensuing in thrombosis (50).

Not all manifestation can be explained by thrombotic events. Although thrombosis underlies many of the neurological complications associated with APL, some others such as migraine, chorea, amaurosis fugax, transverse myelopathy are hardly explained. This implies APL binding to the myelin, cerebral ependyma or choroid epithelium (51, 52). ß2GPI together with anti-ß2GPI has been found to increase permeabilization of giant phospholipid vesicles in vitro. It was suggested that similar permeabilization of the synaptosomes could lead to a (nonthrombotic) defect of a neuronal function (48).

A non-thrombotic mechanism has been suggested for defective placentaion also. It has been linked to a direct APL effect on the trophoblasts without thrombotic phenomena. APL could bind to phosphatidylserine, exposed on the cell surface, during trophoblast differentiation and could affect trophoblast proliferation. ß2-glycoprotein I bound to exposed negatively charged phosphatidylserine appears to act as a bridge between APL and the target tissue (53, 54).

5.4 Unresolved questions

Despite the large number of researchers who are involved in studies of APS and the enormous number of laboratory and clinical reports in the literature, we are still far from a conclusive agreement on the pathogenesis of APS. APL to different plasma proteins and proteins expressed on, or bound to endothelial cells or platelets have been described. They could be the cause, the epiphenomena, or both. On the laboratory level, the inter-laboratory standardization of methods for the detection of APL, determination of antigenic and epitopic specificity of APL to different plasma proteins including their avidity and affinity, cross reactivity to conserved B-cell epitopes, T-cell involvement, are just some of the open questions and proposals for the near future research.

5.5 Take-home messages:

The antiphospholipid syndrome (APS) is an autoimmune-mediated autoimmune disorder in which thromboembolic events (arterial, venous or small vessels thrombosis, recurrent unexplained abortions) occur together with antiphospholipid antibodies (APL). Laboratory diagnostic of APS is based on the detection of the lupus anticoagulant (not only a prolongation of coagulation) or on the detection of antibodies by the standardized ß2-glycoprotein I dependent anticardiolipin antibodies.
Diversity of clinical manifestations and heterogeneity of antiphospholipid antibodies suggest that more than one mechanism is involved in the development of APS.

Thrombotic mechanisms of APS can be grouped as APL interference with haemostatic reaction and APL activation or stimulation of certain cells.

The APL can interrupt protein C pathway on coagulation (acquired resistance to activated protein C, antithrombin III or on fibrinolytic (plasminogen activator) side.

Just some of neurological manifestation of APS could be explained with thrombosis. For others (e.g. migraine, chorea, transverse myelopathy) nonthrombotic mechanisms have been suggested. They include direct interaction of APL with membranes.

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