The Antiphospholipid Syndrome and Endothelial Function

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Patients with the antiphospholipid syndrome have a wide range of clinical findings generally attributable to vascular events in both the arterial and venous circulations, with women also being subject to recurrent miscarriage. Although autoantibodies are termed antiphospholipid antibodies for historical reasons, they are actually directed against phospholipid-binding plasma proteins, specifically β_2-glycoprotein I and prothrombin [1,2].

The tests for aPL fall into two groups. *In vitro* coagulation tests may be prolonged by aPL in which case the aPL is called a lupus anticoagulant. The most commonly used enzyme-linked immunosorbent assay for aPL uses cardiolipin-coated wells and buffer containing bovine serum: this is called the anticardiolipin assay.

The three major clinical features of APS are venous thrombosis, arterial thrombosis, and miscarriage [2]. The sites most commonly affected are the deep pelvic and leg veins, the cerebral arteries (causing stroke), and the cutaneous small vessels (causing painful nodules, ulceration and livido reticularis).

APS and endothelial function

Since veno- and arterial occlusive disorders are relatively common in APS, and immunologic mechanisms are already known to be involved in atherosclerosis and atherothrombosis [3], it is quite logical to assume that patients with an immunologic disorder and a high prevalence of veno- and arterial thrombosis would have an autoimmune disorder that may have an effect on the endothelial cells, and this combined effect causes the clinical outcome of occlusive vascular disorders.

We studied the combined effect of the inflammatory system and the autoimmune system in patients who underwent coronary angioplasty. We followed our patients for 1 year, and evaluated restenosis and the association between restenosis and any of the inflammatory and immunologic markers that were measured on entry to the study. We did not find any correlation between the initial serum amyloid type A and restenosis rate; however, patients whose SAA levels increased by >100% in the first 24 hours and did not develop autoimmune antibodies (anticardiolipin antibodies) had a relative risk of 4.3 for developing restenosis in 1 year [4]. Patients whose SAA levels increased by >100% in the first 24 hours and also had a positive autoantibody had a relative risk of 10.6 for developing restenosis ($P = 0.04$). We previously demonstrated an additive effect between the autoimmune response and the inflammatory response, which was associated with an increased rate of post-balloon percutaneous transluminal coronary angioplasty restenosis [4].

Recently it was found that flow-mediated dilation (endothelium-dependent dilation) was significantly impaired in patients with systemic lupus erythematosus as compared to controls. The nitroglycerin-induced dilation (endothelium-independent dilation) was significantly lower in the anticardiolipin-positive lupus patients when compared to the control group [5].

In lupus, early cardiovascular events in women (age 35–44 years) are increased more than 50-fold [6]. It is possible that this accelerated vascular disease is due to a direct immunologic mechanism. In APS, immunologic mechanisms are involved in promoting atherosclerosis, including the aPL that affect endothelial cells and macrophages as well as cross-reacting with oxidized low density lipoprotein; indeed this syndrome has been described as the "crossroads of autoimmunity and atherosclerosis" [7].

Endothelial function is an important key factor in atherothrombosis and vascular homeostasis

Beta 2 glycoprotein, a major protein co-factor in the APS, has been immunolocalized to human atherosclerotic plaques and co-localized with CD4-positive lymphocytes [8]. Immunization of LDL receptor-deficient and apolipoprotein-E knockout mice (transgenic mice prone to atherosclerosis) with β2GP1 induces early atherosclerosis [9,10]. *In vitro*, macrophage uptake of oxidized LDL is inhibited by β2GP1 in a dose-related manner [11]. Similar findings were demonstrated in cultured endothelial cells, which were recently shown to possess an OxLDL receptor [11]. β2GP1 therefore may have a protective role in atherosclerosis, preventing excessive uptake of LDL by macrophages and the subsequent formation of foam cells central to the formation of atherosclerotic lesions.

aPL = antiphospholipid antibodies
APS = antiphospholipid syndrome
SAA = serum amyloid type A

LDL = low density lipoprotein
β2GP1 = beta 2 glycoprotein
OxLDL = oxidized LDL

Simultaneous addition of β2GP1 and aCL causes increased binding and uptake of OxLDL by macrophages [11]. Monocyte adhesion is increased in endothelial cells cultured with aCL or anti-β2GP1 antibodies when in the presence of β2GP1 [12,13]. *In vitro*, endothelial cells incubated in this way expressed cell surface adhesion molecules, including E-selectin, vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, which were shown to be instrumental in the pathogenesis of atherosclerosis [14].

Patients with insulin-dependent diabetes mellitus are well known to be at high risk for vascular disease, and dysfunction of vascular endothelium is considered to be an early step in the development of diabetic complications. IDDM patients were characterized by significantly increased serum levels of C-reactive protein, polymorphonuclear cell-derived elastase, endothelin-1 and thrombomodulin, while plasma concentrations of fibronectin were significantly decreased, with a significant inverse correlation between endothelin-1 and fibronectin values. Levels of endothelin-1 were directly correlated with von Willebrand factor and anticardiolipin β2GPI. An association between antiphospholipid antibodies and endothelial dysfunction and/or activation is therefore suggested, pointing to a synergism in the early phases of IDDM vascular disease between generation of autoantibodies and endothelial activation [15].

APS is an autoimmune syndrome that is dependent on endothelial cell responses and function

Endothelial cells as a target for aPL

The antiphospholipid syndrome entails a prothrombotic state associated with the presence of cardiolipin antibodies. Recent data suggest that aCL target the plasma co-factor β2GPI rather than negatively charged phospholipids [16]. Another study indicated that anticardiolipin antibodies and anti-endothelial cell antibodies comprise a highly heterogenous population of antibodies with respect to the antigens they recognize, as well as VH gene usage. Monoclonal antibodies from patients with APS do not differ from those derived from normal individuals based on either antigen recognition or VH gene usage. These results suggest the importance of additional predisposing factors in the pathogenesis of the antiphospholipid syndrome [17]. It has been demonstrated that aPL accumulate in late endosomes of human umbilical vein endothelial cells, leading to a redistribution of the cation-independent mannose-6-phosphate receptor. β2GPI was detected at the cell surface and in late endosomes. Incubation of HUVEC with anti-β2GPI antibodies resulted in antibody accumulation at the cell surface and within late endosomes, and in a redistribution of the CI-M6PR from the Golgi apparatus to late endosomes. The accumulation of anti-β2GPI antibodies in late endosomes of endothelial cells and the resulting modification of intracellular protein trafficking may contribute to the pathogenic effects of these antibodies [18].

A recent publication suggests that late endosomes, which are part of the pathway that leads to lysosomes, contain a complex system of poorly characterized internal membranes. These endosomes are therefore known as multi-vesicular or multi-lamellar organelles. These internal membranes contain large amounts of a unique lipid, thus forming specialized domains within endosomes that are involved in sorting the multi-functional receptor for insulin-like growth factor-2 and ligands bearing mannose-6-phosphate, in particular lysosomal enzymes. This unique lipid was demonstrated to be a specific antigen for human antibodies associated with the antiphospholipid syndrome. These antibodies may act intracellularly by altering the protein-sorting functions of endosomes [19].

In a study that explored whether there was evidence of endothelial cell perturbation *in vivo*, serum and plasma were collected from controls and patients with primary APS; and enzyme-linked immunosorbent assays were performed to quantify soluble VCAM-1, soluble ICAM-1, interleukin-6, endothelin-1, von Willebrand factor, and soluble tissue factor. In addition, soluble p-selectin and vascular endothelial growth factor were measured. No significant differences in the levels of blood-borne soluble markers were detected between the patient and control groups except for VEGF and soluble tissue factor, patients having significantly higher levels of VEGF and soluble tissue factor than controls. These results suggest that plasma soluble tissue factor and VEGF may play a role in the pathogenesis of thrombosis in the antiphospholipid syndrome, although the cell of origin of these molecules remains unclear [20].

Summary

Endothelial function and vascular inflammation play major roles in the pathogenesis and clinical outcome of patients with the antiphospholipid syndrome.

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aCL = anticardiolipin antibodies
IDDM = insulin-dependent diabetes mellitus
HUVEC = human umbilical vein endothelial cells
CI-M6PR = cation-independent mannose-6-phosphate receptor
VCAM = vascular cell adhesion molecule
ICAM = intercellular adhesion molecule
VEGF = vascular endothelial growth factor

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Capsule

Mitochondrial protection by frataxin

Friedreich's ataxia is a genetic disorder characterized by a deficiency in frataxin, the mitochondrial iron-binding protein. Bulteau et al. identify a role for frataxin as an iron chaperone protein that is required for the reversible modulation of mitochondrial aconitase activity in response to pro-oxidants. By protecting Fe-S clusters from disassembly, frataxin can prevent iron accumulation and production of the highly reactive and toxic hydroxyl radical. Alterations in the level, structure and chaperone function of frataxin may participate in the progression of degenerative disorders associated with declines in aconitase and mitochondrial activity.

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Capsule

Syphilis and HIV co-infection

HIV and syphilis affect similar patient groups and co-infection is common. Lynn et al. addressed this issue. All patients presenting with syphilis should be offered HIV testing and all HIV-positive patients should be regularly screened for syphilis. Syphilis agent may enhance the transmission of the other, probably through the increased incidence of genital ulcers. Detection and treatment of syphilis can therefore help to reduce HIV transmission. Syphilis may present with non-typical features in the HIV-positive patient: there is a higher rate of symptomless primary syphilis and proportionately more HIV-positive patients present with secondary disease. Secondary infection may be more aggressive and there is an increased rate of early neurologic and ophthalmic involvement. Diagnosis is generally made with serology, but the clinician should be aware of the potential for false-negative serology in both primary and, less commonly, in secondary syphilis. All HIV-positive patients should be treated with a penicillin-based regimen that is adequate for the treatment of neurosyphilis. Relapse of infection is more likely in the HIV-positive patient and careful follow-up is required.

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E. Israeli