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Immunotherapy in antiphospholipid syndrome

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ABSTRACT

Antiphospholipid syndrome (APS) is a disorder characterized by the association of arterial or venous thrombosis and/or pregnancy morbidity with the presence of antiphospholipid antibodies (anticardiolipin antibodies, lupus anticoagulant antibodies, and/or anti-β2-glycoprotein I antibodies).
Thrombosis is the major manifestation in patients with aPLs, but the spectrum of symptoms and signs associated with aPLs has broadened considerably, and other manifestations, such as thrombocytopenia, non-thrombotic neurological syndromes, psychiatric manifestations, livedo reticularis, skin ulcers, hemolytic anemia, pulmonary hypertension, cardiac valve abnormality, and atherosclerosis, have also been related to the presence of those antibodies.
Several studies have contributed to uncovering the basis of antiphospholipid antibody pathogenicity, including the targeted cellular components, affected systems, involved receptors, intracellular pathways used, and the effector molecules that are altered in the process.
Therapy for thrombosis traditionally has been based on long-term oral anticoagulation; however, bleeding complications and recurrence despite high-intensity anticoagulation can occur. The currently accepted first-line treatment for obstetric APS (OAPS) is low-dose aspirin plus prophylactic unfractionated or low-molecular-weight heparin (LMWH). However, in approximately 20% of OAPS cases, the final endpoint, i.e. a live birth, cannot be achieved.
Based on all the data obtained in different research studies, new potential therapeutic approaches have been proposed, including the use of new oral anticoagulants, statins, hydroxychloroquine, coenzyme Q10, B-cell depletion, platelet and TF inhibitors, peptide therapy or complement inhibition among others. Current best practice in use of these treatments is discussed.

1. Pathogenic mechanisms in the antiphospholipid syndrome

1.1. Activation of tissue factor and related pro-thrombotic molecules

Many mechanisms have been proposed to explain the thrombotic tendency of patients with APS, but the pathogenesis seems to be multifactorial. Procoagulant cell activation, accompanied by tissue factor (TF) expression and TF pathway upregulation, is one of the key events in the pathophysiology of thrombosis in patients with APS. Previous studies showed elevated plasma levels of soluble TF in APS patients, and thereafter we reported that monocytes isolated from APS patients had high TF expression [1–3]. At the molecular level, the signal transduction mechanisms induced by aPLs have been explored. An ex-vivo study led us to show that aPLs induced TF in monocytes from APS patients by activating – simultaneously and independently – the phosphorylation of mitogen-activated protein kinase (MAPK)/extracellular regulated kinase protein, and the p38 MAPK-dependent nuclear translocation and activation of nuclear factor-κB (NF-kB)/Rel proteins [4]. Similar results have been reported in platelets, monocyte cell lines, and in vivo models of aPL-induced thrombogenicity [5–7]. Parallel studies performed in endothelial cells (ECs) further concluded that: 1) NF-κB plays an essential role in TF activation induced by aPLs [8]; and 2) p38 MAPK phosphorylation and NF-κB activation are involved in the aPL-induced increase in TF transcription, function, and expression; interleukin (IL)-6 and IL-8 upregulation; and inducible nitric oxide synthase expression [9]. Previous reports indicate a close relationship between TF and vascular endothelial growth factor (VEGF), a family of proteins involved in normal vascular development and in relevant pathophysiologic settings, including cancer, wound healing, and inflammation [10]. Precedent studies had reported increased plasma levels of VEGF in APS patients [11]. Then we analyzed the VEGF and FMS-related tyrosine kinase 1 (FLT1) expression levels in monocytes of APS

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patients, the molecular mechanisms involved in their aPL-induced expression, and their association with the elevated TF expression found in these patients [12]. Our data primarily showed that monocytes from APS patients expressed increased levels of both VEGF and FLT1 in comparison with monocytes from healthy donors. Furthermore, in vitro results indicated that this cytokine was produced by monocytes when treated with aPLs, and that the p38 MAPK signaling pathway played an important role. Thus, VEGF might act as a regulatory factor in aPL-mediated monocyte activation and TF expression, thereby contributing to the proinflammatory–prothrombotic phenotype of APS patients. Moreover, the excess of plasmatic thrombin in APS, most likely induced by TF expression, and acting through the activation of protease activated receptors (PARs 1 and 2, also increased in monocytes of APS [13]), might also be related to the elevated VEGF production found in that patients.

The application of proteomic techniques to APS patients' monocytes has led one to identify an altered expression of proteins. This abnormal expression might be directly related to the pathogenic mechanisms of APS. Our group has addressed the question of predicting thrombotic risk in APS patients by using a proteomic approach on purified human monocytes [14]. The proteins identified as more significantly deregulated in the monocytes from patients with APS and thrombosis were annexin A1 (AnxA1), annexin A2 (AnxA2), ubiquitin Nedd8, Rho A protein, protein disulfide isomerase (PDI) and Hsp60. These proteins have been shown to be associated with the induction of a procoagulant state, as well as autoimmune-related responses. In that way, Anx A2 has recently been directly involved in the pathogenesis of APS. It has been demonstrated that binding of β2GPI to human umbilical vein endothelial cells is mediated by Anx A2 [15]. Furthermore, thrombosis and TF upregulation are significantly decreased in Anx A2 deficient mice in vivo. By functioning as a receptor for β2GPI, Anx A2 is a target not only for anti-Anx A2 antibodies but also for anti-β2GPI antibodies, which are direct inductors of TF. These data suggest that Anx A2 might constitute a common receptor for aPL induction of monocyte activation [16]. Protein disulfide isomerase (PDI) has been also demonstrated to be linked to TF on the cell surface when coagulant activity is low and TF-VIIa signaling is enabled. Moreover, PDI expression reduction has been associated with a two-fold increase of TF procoagulant activity [17]. Overexpression of PDI suppresses NF-κB-dependent transcriptional activity [18]. As the aberrant activation of the NF-κB signaling pathway is likely to contribute to the development of APS, the decrease expression of this protein might be related to the constitutive activation of this transcription factor in the APS. In addition, as described below, the PDI has a crucial role in development of the immunogenic form of β2GPI in the setting of APS.

1.2. Atherogenesis

Experimental studies and human observations suggest that APS is associated with atherosclerosis. In fact, innate and adaptive immune responses participate in the pathogenesis of both diseases. Anti-oxLDL, anti-aPL, anti-β2GPI, and anti-HSP antibodies, among others, have been found in patients with APS and atherosclerosis [19]. Endothelial dysfunction, oxidative stress, an increase in cell adhesion molecules, and active platelets are common findings in both diseases. In addition, macrophages, dendritic cells, T-cell activation, and CD40–CD40 ligand interaction are considered pathogenic mechanisms of atherosclerosis and APS [20,21].

Notably, aPL antibodies trigger an inflammatory cascade, and they have been associated with atherosclerosis as well as cerebrovascular and peripheral arterial diseases [22,23]. Moreover, aPL antibodies may cross-react with oxidized low-density lipoproteins (ox-LDLs), and both aPL and anti-ox-LDL antibodies have been implicated in the pathogenesis of atherosclerosis associated with systemic lupus erythematosus (SLE) and APS. It has been shown that aPL antibodies, in particular anti-β2GPI antibodies, can accelerate the influx of ox-LDLs into macrophages [24]. Other autoantibodies, such as anti-high-density lipoproteins (HDLs) and antiapolipoprotein A-I, also have been detected in APS. In addition, macrophages and ECs bind to β2GPI during the atherosclerotic process. In this regard, anticardiolipin (aCL) antibodies can induce monocyte adherence to ECs, which is mediated by adhesion molecules such as ICAM-1, VCAM-1, and E-selectin. Thus, aCL antibodies might promote atherosclerosis by attracting monocytes into the vessel wall. Moreover, a correlation between serum levels of aCL and anti-β2GPI antibodies and the incidence and severity of acute coronary syndrome, myocardial infarction, and stroke have been demonstrated previously [25,26].

Early endothelial dysfunction [27] and increased carotid intima–media thickness also have been observed in APS [28]. Accordingly, in a recent study, within a cohort of 43 APS patients, we found that patients with higher aPL-IgG titers showed a strong association with the development of thrombotic events and also with the increased intimate–media thickness (IMT) of the carotid arteries [29]. The issue of early atherosclerosis development in APS patients has shown controversial data in past years. In our series, the presence of plaques in carotid arteries in a significant number of APS patients was in favor of the evidence of an accelerated atherosclerosis. Our results confirmed previous reports showing greater IMT in APS, related to the titer of aPL-IgG [30–32]. Moreover, our data pointed to the existence of premature atherosclerosis as a clinical feature of thrombotic APS patients, so that in our series, 11 of 12 of the APS patients who presented increased IMT values had suffered at least 1 thrombotic event. In addition, our results agreed with a recent study showing that premature atherosclerosis, as defined by IMT, occurs in thrombotic APS over 30 years [28]. Premature atherosclerosis might be facilitated by the existence of an inflammatory status in APS, which seems not to be coordinated by "classic" cytokines such as TNFα or IL-6 but by other known inflammatory mediators, including VEGF and tPA, as well as various chemokines (IL-8, MCP-1, or MIP-1α) whose main function is to recruit, e.g., neutrophils, monocytes, B cells, and T helper cells to the sites of inflammation [33,34]. Thrombus formation is a key event in the development of the intimae thickening, considered to comprise the early stage of atherosclerosis plaque formation. Many studies have demonstrated that TF is present in atherosclerosis lesions and contributes to atherogenesis [35]. TF mediates the responses that are critical for hemostasis and thrombosis, as well as inflammatory reactions. Thus TF, whose expression is also significantly increased in monocytes of APS patients, together with factors downstream of the coagulation cascade and the PAR2 activation system, would act as an additional multifactorial regulator of atherogenesis.

1.3. Oxidative stress and mitochondrial dysfunction

Various studies have evidenced that oxidative stress is directly involved in the pathophysiology of both APS and SLE. Mitochondrial dysfunction, accompanied with ATP depletion, oxidative stress, abnormal activation, and death signal processing in lupus T cells have been demonstrated previously [36]. In the setting of APS, aCL antibodies seem to play an important role in the oxidative status by inducing nitric oxide (NO) and superoxide production, resulting in enhanced levels of plasma peroxynitrite, a powerful pro-oxidant substance [37]. Titers of aCL antibodies have been found positively correlated to plasma levels of F2-isoprostanes, sensitive markers of in vivo lipid peroxidation, indicating enhanced oxidative stress in APS [38,39]. Functional and structural arterial abnormalities have been associated with lower activity of paraoxonase, an antioxidant enzyme linked to HDLs that prevents LDL oxidation. Moreover, in patients with aPL antibodies, HDL reduced NO bioavailability and showed impaired anti-inflammatory and antioxidant properties [40]. Thus, there is substantial evidence showing oxidative damage to lipids and proteins in APS. In a very recent study [29] we showed an increased production of reactive oxygen species (ROS) by monocytes and neutrophils that disturbs the redox status and in turn may influence the expression of prothrombotic and proinflammatory

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