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Review

Anti-phosphatidylethanolamine antibody, thromboembolic events and the antiphospholipid syndrome

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ABSTRACT

The antiphospholipid syndrome (APS) is an acquired disorder characterized by arterial and/or venous thrombosis and pregnancy morbidity. In solid assays, sera from patients with APS usually react to negatively charged phospholipids (PL) and PL cofactors such as β2-glycoprotein I (β2GPI). Binding to neutral PL such as phosphatidylethanolamine (PE) is less common. PE is one of the main lipid components of the biological membranes, being mostly located in the inner leaflet. In 1989 we reported the first case of primary APS whereby a LA was accompanied not by an anticardiolipin antibody (aCL), but by an antibody to PE (aPE). In this review, we update the literature concerning the presence of aPE in patients with thromboembolic events and obstetric morbidity. We also discuss aPE as the sole antibody detected in many of these clinical circumstances. An eventual link of aPE antibodies with failure of in vitro fertilization is also considered as well as uncommon clinical associations of aPE that are also discussed.

Contents

1. Introduction

The antiphospholipid syndrome (APS) is a systemic thrombotic diathesis of young adults. The mechanisms by which antiphospholipid antibodies (aPL) cause thrombosis and obstetric morbidity are poorly understood, but probably include inhibition of natural anticoagulants, activation of platelets and endothelial cells, blocking of the fibrinolytic system, and triggering of the complement cascade [1]. A 'second hit', frequently linked to innate inflammatory responses, is probably necessary to initiate the thrombotic vasculopathy characteristic of APS [2].

It is well known that aPL from patients with APS preferentially target negatively charged phospholipids (PL) and/or their complex with plasma proteins such as β2-glycoprotein I (β2GPI) [3].

Binding of APS sera to zwitterionic compounds such as phosphatidylethanolamine (PE) was not recognized by the time aPL specificities were detailed by Gharavi et al. in 1987 [4]. PE, a neutral PL in the pH range 2 to 7, is the main lipid component of the microbial membranes, and it is largely found in mitochondria. The PE molecule consists of a combination of glycerol esterified with two fatty acids and phosphoric acid. The phosphate group (negatively charged) is bound to ethanolamine, an alcohol with a positive charge. These contrasting charges characterize PE as a typical zwitterionic PL. In biological membranes, PE seems to work as a "chaperone" to guide the folding of other membrane proteins; it also supports active transport by the lactose permease [5].

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In *Bacillus megaterium*, PE is asymmetrically distributed in the cell membrane, with one-third in the outer leaflet and two-thirds in the inner leaflet. This asymmetric distribution of PE is usual in the structure of biological membranes [6]. PE structure and main location in a hypothetical lipid bilayer are shown in Fig. 1.

As PE molecules are mainly located in the inner leaflet of biological membranes [6,7], their bioavailability as targets for aPE at the endothelial cell surface is an issue to be clarified; it is possible that a primary event in APS patients expose PE molecules in the outer part of the membrane, making possible for the antibody to bind. Recent experiments utilizing rat aortic arch showed high levels of PE distributed along the endothelial surfaces, where the luminal location of PE might relate to aPE autoimmunity and thrombotic risk [7].

In 2007, it was shown that human monocytes and platelets, when activated, generated four analogous PE lipids that contained 12 or 15-hydroxyeicosatetraenoic acid (HETE); these PE-esterified HETEs, within the immune cells, could be signaling the lipoxygenase cascade [8]. Whether such proinflammatory effects attributable to PE are playing a role in systemic disorders as APS is an open question.

2. Pathogenesis and laboratory aspects

Although still controversial, the interest for aPE antibodies dates back to 1989, when we described for the first time a case of primary APS whereby a lupus anticoagulant (LA) was accompanied only by an IgM aPE. The aPE ELISA results were confirmed by inhibition studies and affinity purification [9]. Six other patients with LA but no anticardiolipin (aCL), showed no binding to various negatively charged compounds or PE. However, the LA activity could be removed by hexagonal phase PE [10], a technique reported by Rauch et al. in 1986 [11]. A later study dated 1988 demonstrated that PE extracted from platelet membranes inhibited aCL activity better than any other PL, even though the great majority of aCL-positive sera did not bind PE in solid assays [12].

Interestingly, plasma reactivity to hexagonal phase PE was more consistently associated with LA than with aPE antibodies [13]. Due to the ability of the hexagonal phase PE to inhibit the prolongation of the clotting time, several groups suggested that LA might represent a subset of aPE. While some aPE appears to be independent of known PE-binding plasma proteins, others depend on the presence of kininogens and even prothrombin [14]. Kininogens and/or high molecular weight kininogen binding proteins were shown to serve as a "cofactor" significantly more often for IgG than for IgM aPE [15].

PE was found to induce specific conformational changes in the kininogens recognizable by aPE antibodies [16]. aPE antibodies bound kininogen domain 3 [17], and autoantibodies to kininogen–PE complexes enhanced thrombin-induced platelet aggregation [18]. Some other aPE antibodies appeared to recognize factor XI and prekallikrein independently or in addition to the kininogens [19]. In obstetric APS, disruption of the kallikrein–kinin system by aPE antibodies might play a pathogenic role in early pregnancy losses [20].

In solid assays, the structure of PE is basically lamellar. A modified ELISA for detecting IgG aPE antibody was reported as a valuable method for diagnosis and prognosis of patients with recurrent fetal loss in 1990 [21]. In 1999, flow cytometry with appropriate quantification was described as a sensitive aPE assay in APS patients [22]. More recently, aPE reactivity in ELISA plates was shown to be dependent on the lipid concentration of the buffer component [23]. In LA-positive plasma, detection of aPE antibodies in solid assays varied according to the microtitre plate utilized [24]. In ELISA plates, cryoprecipitation did not seem to interfere significantly with the levels of IgM aPE [25]. As a whole, the aPE ELISA still lacks international standardization.

In a recent study, mice passively immunized with aPE showed impairment of trophoblast giant cell invasion and increased placental

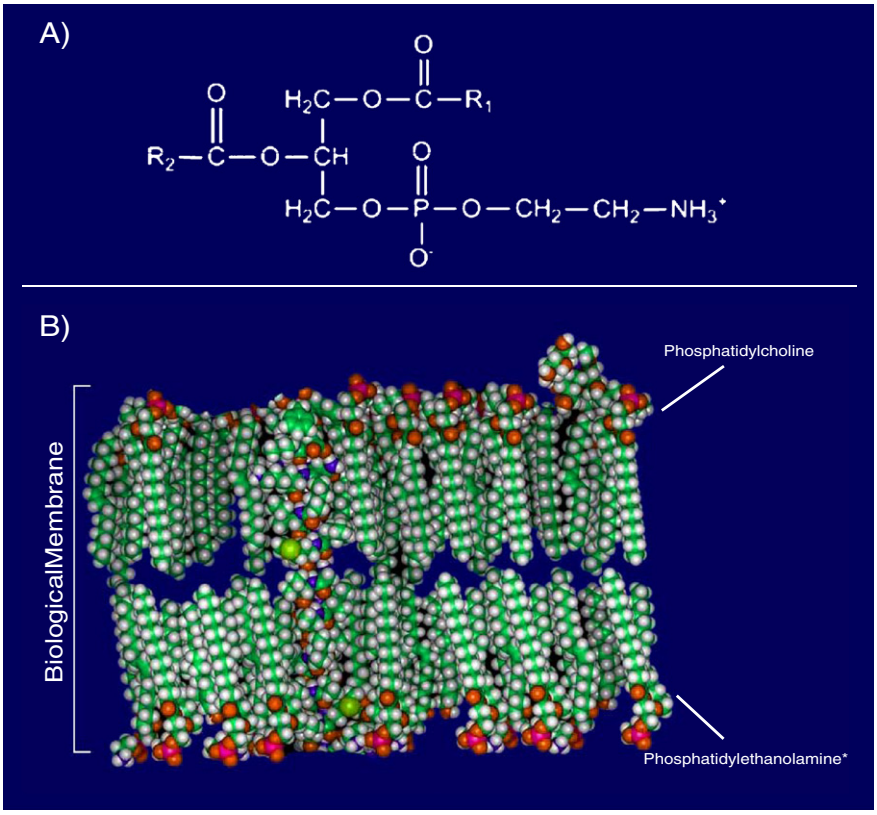

Fig. 1. A) Molecular structure of phosphatidylethanolamine (PE) showing a glycerol esterified with two fatty acids (R1 and R2); the negatively charged phosphate group binding to the positively charged ethanolamine characterizes PE as a zwitterionic phospholipid. B) Main location of PE* in the inner aspect of a lipid bilayer, as opposed to the predominantly outer position of phosphatidylcholine.