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Impaired Fibrinolysis in the Antiphospholipid Syndrome

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Abstract The antiphospholipid syndrome (APS) is characterized by venous and/or arterial thrombosis, or recurrent fetal loss, in the presence of antiphospholipid antibodies (APL). The pathogenesis of APS is multifaceted and involves numerous mechanisms including activation of endothelial cells, monocytes, and/or platelets; inhibition of natural anticoagulant pathways such as protein C, tissue factor inhibitor, and annexin A5; activation of the complement system; and impairment of the fibrinolytic system. Fibrinolysis—the process by which fibrin thrombi are remodeled and degraded—involves the conversion of plasminogen to plasmin by tissue plasminogen activator (tPA) or urokinase-type plasminogen activator, and is tightly regulated. Although the role of altered fibrinolysis in patients with APS is relatively understudied, several reports suggest that deficient fibrinolytic activity may contribute to the pathogenesis of disease in these patients. This article discusses the function of the fibrinolytic system and reviews studies that have reported alterations in fibrinolytic pathways that may contribute to thrombosis in patients with APL. Some of these mechanisms include elevations in plasminogen activator inhibitor-1 levels, inhibitory antibodies against tPA or other components of the fibrinolytic system, antibodies against annexin A2, and finally, antibodies to β_2-glycoprotein-I (β_2GPI) that block the ability of β_2GPI to stimulate tPA-mediated plasminogen activation.

Keywords Fibrinolysis · Beta2-glycoprotein I · Antiphospholipid · Plasminogen · Fibrin · t-PA

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Introduction

The antiphospholipid syndrome (APS) is characterized by venous and arterial thrombosis and/or pregnancy loss in the presence of antiphospholipid antibodies (APL) [1]. APL are the most common cause of acquired thrombophilia and are significantly associated with cardiovascular death following an initial thrombotic event [2]. However, the term *antiphospholipid antibody* is misleading because the majority of APL associated with APS are directed against phospholipid-binding proteins such as β_2-glycoprotein-I (β_2GPI) and prothrombin. In the clinical laboratory, several types of APL may be distinguished; lupus anticoagulants are detected by their ability to prolong phospholipid-dependent coagulation tests such as the dilute Russell's viper venom time [3], whereas anticardiolipin antibodies (ACA) or anti-β_2GPI antibodies are detected using enzyme linked immunosorbent assay. However, although APL, particularly lupus anticoagulants and anti-β_2GPI antibodies, are associated with an increased risk of thrombosis, none of the currently available clinical assays can predict with precision which patients with APL are destined to develop thrombotic events [4–6].

The pathogenesis of APL-mediated thrombosis likely involves several pathogenic mechanisms such as the activation of endothelial cells, monocytes, and/or platelets

and the effects of thrombogenic microparticles derived from these cells [7]. Other mechanisms include inhibition of the activity of natural antithrombotic proteins, such as protein C, tissue factor pathway inhibitor, and annexin A5, activation of the complement system, and impairment of fibrinolysis, which regulates thrombus remodeling and dissolution. Although acknowledging the importance of impaired anticoagulant activity in the APS, this review focuses on the potential contribution of deficient fibrinolysis to this disorder.

Components of the Fibrinolytic System

Fibrinolysis is a tightly regulated process by which a fibrin-rich thrombus is remodeled and degraded. The activation of the fibrinolytic system results in the conversion of the proenzyme plasminogen to the proteolytic enzyme plasmin. Plasmin degrades fibrin thrombi, resulting in the release of a variety of fibrin degradation products, including the fibrin D-dimer. Plasminogen activation is mediated by one of two plasminogen activators, tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). tPA is synthesized by vascular endothelial cells and macrophages, can be demonstrated in nearly all tissues except liver, and is thought to have an important role in the expression of vascular wall fibrinolytic activity and resolution of thrombi. In addition to tPA, uPA has been well characterized, although it is generally considered more important in extravascular processes such as cellular invasion and migration [8, 9].

Plasmin is generated through both an extrinsic pathway in which activation is mediated by plasminogen activators such as tPA and uPA, as well as through an intrinsic pathway, in which activation occurs through contact system components such as factor XII [8, 10]. The level of tPA in plasma is normally low, but can be increased by stress and exercise. The activation of plasminogen by tPA is dramatically accelerated in the presence of fibrin, which contains binding sites for both enzyme and substrate, and lowers the K_M for their interaction [9]. Once fibrin-bound plasmin has been generated, it is partially protected from its plasma inhibitors, α_2-macroglobulin and α_2-plasmin inhibitor, and thus able to degrade fibrin, resulting in clot lysis and the release of various fibrin degradation products that may have additional effects on vascular function [9].

The activity of tPA is primarily regulated by the serine protease inhibitor, plasminogen activator inhibitor type 1 (PAI-1) [11], although a second inhibitor (plasminogen activator inhibitor type 2; PAI-2) may be present at significant concentrations during situations such as pregnancy [12]. Fibrinolysis is tightly regulated through the concentrations of the activator enzyme, as well as the inhibitor at specific sites.

Cell surfaces also play a critical role in the regulation of plasminogen activator activity through the expression of proteins that bind and modulate the activity of components of the plasminogen activator system. One such protein that plays a critical role in the regulation of plasminogen activation on endothelial cells is annexin A2. Experimental evidence has shown that annexin A2 acts as a cell surface coreceptor for plasminogen and tPA, bringing the two reactants into close spatial approximation and stimulating the activation of plasminogen by tPA [13]. The efficiency of this interaction is augmented by the presence of paired p11 proteins as a component of the annexin A2 heterotetramer [14]. The importance of annexin A2 in this regard is demonstrated in a report by Ling et al. [15] in which annexin A2—deficient mice demonstrated delayed clearance of arterial thrombi after injury, increased fibrin deposition in microvessels, and significantly reduced plasmin generation at the endothelial cell surface. Moreover, Ishii et al. [16] reported that pretreatment of rats with annexin A2 prevented carotid artery thrombosis in response to injury.

Because of the central role of plasmin in the remodeling and degradation of the fibrin thrombus, and tight regulation of the conversion of plasminogen to plasmin, disruption of either of these processes may be prothrombotic. This indeed may occur in some patients with APS, and may be an important mechanism predisposing such patients to thrombosis.

Impairment of Fibrinolysis by APL: Mechanisms

As discussed above, impairment of fibrinolysis due to APL may contribute to the development of thrombosis [17, 18]. Several studies have assessed the effects of APL on the activity of the fibrinolytic system. Jurado et al. [19] found increased PAI-1 activity after venous occlusion in patients with connective tissue disorders, including APS, compared with healthy controls; however, the extent of these increases did not correlate with APL levels or a history of thrombosis. Ames et al. [20] also reported that patients with APS displayed elevated PAI-1 levels and decreased tPA release after venous occlusion. These studies suggest that both asymptomatic patients with APL, as well as patients with APS, may have impaired fibrinolysis [20].

Another potential mechanism for impaired fibrinolysis in patients with APL is suggested by the findings of Atsumi et al. [21], who reported elevated levels of lipoprotein (a) [Lp(a)] in the plasma of patients with APL. Lp(a), which shares structural homology with plasminogen through the expression of kringle domains homologous to those in plasminogen, competes with plasmin(ogen) for binding to fibrin, and thus interferes with plasmin-mediated fibrin degradation. These authors also suggest that Lp(a) might also interact with cellular plasminogen receptors [21], and others have

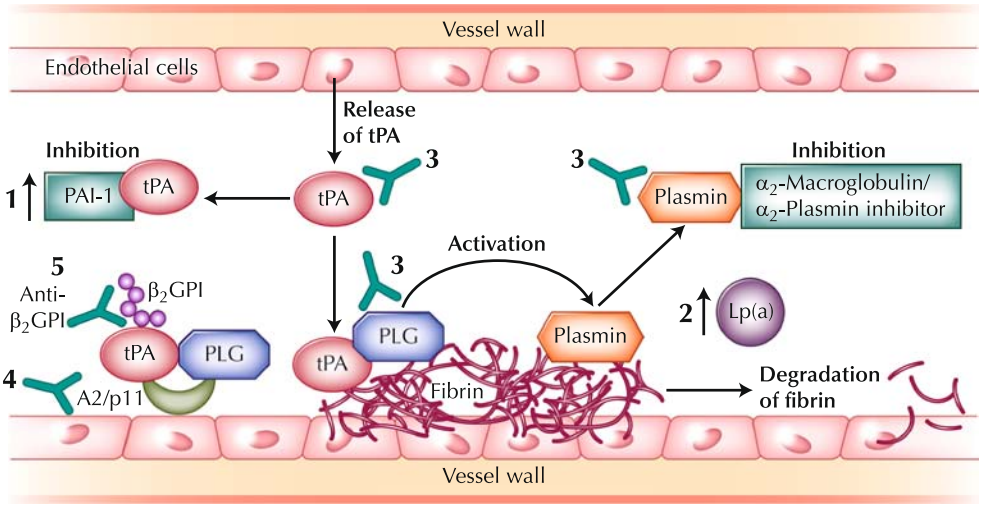

Fig. 1 Overview of fibrinolysis and the mechanisms by which fibrinolysis may be impaired in antiphospholipid syndrome (APS). Increased plasminogen activator inhibitor type 1 (PAI-1) activity in patients with APS may inhibit tissue plasminogen activator (tPA) activity (*1*). Elevated lipoprotein (a) (Lp[a]) has been observed in some patients with APS; Lp(a) shares structural similarity to plasminogen (PLG) and competes with PLG/plasmin for fibrin binding (*2*). **A**ntibodies to tPA, PLG, or plasmin may inhibit fibrinolysis (*3*). Antibodies to annexin A2 block the ability of annexin A2 to promote tPA-mediated PLG activation (*4*). Antibodies to β_2-glycoprotein-I (β_2GPI) interfere with the interaction of β_2GPI with tPA and enhancement of tPA activity (*5*)

demonstrated that Lp(a) may increase PAI-1 expression by endothelial cells [22].

More recent studies have indicated additional mechanisms by which APL may lead to the impairment of fibrinolytic activity (Fig. 1). Some APL have been reported to bind plasmin and inhibit fibrinolysis, suggesting that plasmin may be an autoantigen in APS [23••, 24]. Another study by Chen et al. [23••] using monoclonal antibodies obtained from mice immunized with human plasmin supports this observation. Indeed, Yang et al. [24] reported that 28% of patients with APL had antiplasmin antibodies that could block plasmin-mediated fibrinolysis. Others reported that some APL bind tPA and block its ability to activate plasminogen [23••, 25, 26], findings supported by observations that found an inverse correlation between the presence of anti-tPA antibodies in patients with APS and net plasma tPA activity [26].

Although these studies provide insight into the potential interactions of APL with the plasminogen activator system, they do not directly address the role of β_2GPI. β_2GPI is the major target antigen in patients with APS [27]. Its role in mediating the interactions of APL with anionic phospholipids, as well as cell surfaces, has been demonstrated [28, 29••]. However, recent studies have suggested that β_2GPI and β_2GPI-reactive antibodies may have direct interactions with the fibrinolytic system.

β_2GPI and Impaired Fibrinolysis in APS

β_2GPI, also referred to as apolipoprotein H, is an abundant phospholipid-binding protein, which circulates in the plasma at a concentration of about 4 μM. β_2GPI belongs to the complement control protein superfamily and contains five short consensus repeats (SCR). Domain V of β_2GPI is an atypical SCR that contains a lysine-rich sequence that facilitates the binding of β_2GPI to phospholipids [30]. Plasmin cleaves this sequence, leading to a loss of phospholipid binding. Recent studies suggest that β_2GPI may also interact directly with components of the plasminogen activator system and affect the generation of cell surface and plasma fibrinolytic activity.

Ieko et al. [18] used a chromogenic assay to demonstrate that β_2GPI blocks the neutralization of tPA activity by PAI-1 in a concentration-dependent manner. The addition of IgM APL blocked this activity, allowing the full inhibitory effect of PAI-1 on tPA activity to be realized. In another study, Takeuchi et al. [17] developed a chromogenic assay to assess intrinsic fibrinolytic activity in euglobulin fractions. Monoclonal anti-β_2GPI antibodies in the presence of β_2GPI reduced intrinsic fibrinolytic activity in the euglobulin fractions, even in the presence of excess activated coagulation factor XII. Taken together, these data suggest that APL may inhibit both intrinsic and extrinsic fibrinolytic pathways in a β_2GPI-dependent manner.

β_2GPI may also regulate fibrinolysis through direct interactions with plasminogen. β_2GPI domain V can be proteolytically cleaved by plasmin to create "nicked" β_2GPI, which has a lower affinity for anionic phospholipid than intact β_2GPI [31]. In one report, Yasuda et al. [32] demonstrated that nicked, but not intact β_2GPI, binds plasminogen and blocks the formation of plasmin by tPA. These authors proposed that the binding interaction occurs

between a lysine-rich sequence in domain V of nicked β_2GPI and a lysine binding site in kringle domain V of plasminogen. This report suggests that nicked β2GPI may control extrinsic fibrinolysis through a negative feedback mechanism [32].

In contrast, a more recent study by Lopez-Lira et al. [33] suggests that intact β_2GPI may promote plasmin generation. These authors found that low-affinity binding of intact β_2GPI to plasminogen was mediated by lysine residues to form a ternary complex with tPA. In the presence of plasminogen and tPA, a β_2GPI-dependent increase in plasmin generation was observed [33]. Taken together with the results of Yasuda et al. [32], these findings suggest that intact β_2GPI may stimulate fibrinolysis, but that following plasminogen activation and cleavage of β_2GPI, the cleaved molecule may inhibit further plasmin generation.

Our laboratory has identified a high affinity of interaction between β_2GPI and tPA that led to marked enhancement of the amidolytic activity of tPA, as well as its ability to stimulate fibrin degradation in the presence of plasminogen. This interaction was at least partially mediated through domain V of β_2GPI, and enhanced the efficiency of tPA-mediated plasminogen activation (K_{cat}/K_M) by about 20-fold. Additional studies demonstrated that clot lysis was delayed in plasma that had been depleted of β_2GPI, and that restoration of β_2GPI normalized clot lysis times, suggesting that β_2GPI plays a role in stimulating fibrinolysis in plasma, and not just in the presence of isolated components of the plasminogen activator system. Importantly, the addition of IgG from the serum of patients with APS and anti-β_2GPI antibodies, as well as monoclonal anti-β_2GPI antibodies, significantly impaired β_2GPI-mediated enhancement of fibrinolysis [34••]. These results suggest that β_2GPI-reactive APL might impair fibrinolysis by blocking the ability of β_2GPI to stimulate clot lysis, thus promoting thrombus growth and stability.

Finally, Lázaro et al. [35] incubated human umbilical vein endothelial cells with plasma obtained in the third trimester from normal pregnant women. When monoclonal IgM ACA cloned from a patient with APS were present during the incubation, subsequent analysis of the supernatants revealed increased levels of factor VIIa and decreased amounts of two-chain (active) urokinase compared with control incubations that included a monoclonal IgM ACA from a patient without APS. The mechanisms underlying this interesting observation have yet to be determined [35].

APL and Annexin A2-Mediated Fibrinolysis

Previous work from our laboratory has defined annexin A2 as an endothelial receptor for β_2GPI that mediates endothelial cell activation by anti-β_2GPI antibodies [36]. However, as noted above, annexin A2 also stimulates fibrinolysis through binding of tPA and plasminogen [37, 38]. Interestingly, in addition to anti-β_2GPI antibodies, antibodies that react directly with annexin A2 and correlate with a history of thrombosis have been reported in patients with APS [39]. These antibodies inhibited the ability of annexin A2 to potentiate tPA-mediated plasminogen activation, and blocked annexin A2—dependent generation of plasmin on the endothelial cell surface in the presence of tPA. These antibodies also promoted thrombosis in a murine model, causing a significantly increased thrombus size in wild-type mice compared with mice deficient in annexin A2. Thus, it appears likely that in some individuals with APS, anti-annexin A2 antibodies inhibit cell-surface fibrinolysis through direct interactions with endothelial cell annexin A2.

Conclusions

The pathogenesis of thrombosis in patients with APL is multifactorial. APL promote thrombosis through interference with a number of naturally occurring anticoagulant pathways, such as activated protein C and annexin A5, and may also disrupt fibrinolysis. Because β_2GPI is the major target of APL antibodies, and plays an important role in facilitating fibrinolysis, it is not surprising that anti-β_2GPI antibodies are likely to be important in the disruption of natural fibrinolytic activity. Based on work conducted in our laboratory and others, it appears that β_2GPI may act as a scaffolding protein for stable assembly of profibrinolytic complexes, ultimately promoting thrombus remodeling and degradation. Therefore, additional characterization of the interactions of β_2GPI and anti-β_2GPI antibodies with the fibrinolytic system may yield targets for therapeutic intervention in the APS.

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References

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance

1. Eby C: Antiphospholipid syndrome review. Clin Lab Med 2009, 29:305–319.
2. Ozturk MA, Haznedaroğlu IC, Turgut M, Goker H: Current debates in antiphospholipid syndrome: the acquired antibody—mediated thrombophilia. Clin Appl Thromb Hemost 2004, 10:89–126.
3. Brandt JT, Triplett DA, Alving BA, Scharrer I: Criteria for the diagnosis of lupus anticoagulants: an update. Thromb Haemost 1995, 74:1185–1190.

4. Galli M, Borrelli G, Jacobsen EM, et al.: Clinical significance of different antiphospholipid antibodies in the WAPS (warfarin in the antiphospholipid syndrome). Blood 2007, 110:1178–1183.
5. Forastiero R, Martinuzzo M, Pombo G, et al.: A prospective study of antibodies to B2 glycoprotein I and prothrombin, and risk of thrombosis. J Thromb Haemost 2005, 3:1231–1238.
6. Bizzaro N, Ghiradello A, Zampieri S, et al.: Anti-prothrombin antibodies predict thrombosis in patients with systemic lupus erythematosus: a 15-year longitudinal study. J Thromb Haemost 2007, 5:1158–1164.
7. Pierangeli SS, Chen PP, Raschi E, et al.: Antiphospholipid antibodies and the antiphospholipid syndrome: pathogenic mechanisms. Semin Thromb Hemost 2008, 34:236–250.
8. Zorio E, Gilabert-Estellés J, España F, et al.: Fibrinolysis: the key to new pathogenetic mechanisms. Curr Med Chem 2008, 15:923–929.
9. Castellino FJ, Ploplis VA: Structure and function of the plasminogen/plasmin system. Thromb Haemost 2005, 93:647–654.
10. Renne T, Gailani D: The role of factor XII in hemostasis and thrombosis: clinical implications. Expert Rev Cardiovasc Ther 2007, 5:733–741.
11. Huber K: Plasminogen activator inhibitor type-1 (part one): basic mechanisms, regulation, and role for thromboembolic disease. J Thromb Thrombolysis 2001, 11:183–193.
12. Medcalf RL, Stasinopoulos SJ: The undecided serpin. The ins and outs of plasminogen activator inhibitor type 2. FEBS J 2005, 272:4858–4867.
13. Hajjar KA: The endothelial cell tissue plasminogen activator receptor. J Biol Chem 1991, 266:21962–21970.
14. Kwon M, MacLeod TJ, Zhang Y, et al.: S100A10, annexin A2, and annexin a2 heterotetramer as candidate plasminogen receptors. Front Biosci 2005, 10:300–325.
15. Ling Q, Febbraio M, Deora B, et al.: Annexin II is a key regulator of fibrin homeostasis and neoangiogenesis. J Clin Invest 2004, 113:38–48.
16. Ishii H, Yoshida M, Hiraoka M, et al.: Recombinant annexin II modulates impaired fibrinolytic activity in vitro and in rat carotid artery. Circ Res 2001, 89:1240–1245.
17. Takeuchi R Atsumi T, Ieko M, et al.: Suppressed intrinsic fibrinolytic activity by monoclonal anti-beta-2 glycoprotein I autoantibodies: possible mechanism for thrombosis in patients with antiphospholipid syndrome. Br J Haematol 2002, 119:781–788.
18. Ieko M, Ichicawa K, Atsumi T, et al.: Effects of beta 2-glycoprotein I and monoclonal anticardiolipin antibodies on extrinsic fibrinolysis. Semin Thromb Haemost 2000, 26:85–90.
19. Jurado M, Páramo JA, Gutierrez-Pimentel M, Rocha E: Fibrinolytic potential and antiphospholipid antibodies in systemic lupus erythematosus and other connective tissue disorders. Thromb Haemost 1992, 68:516–520.
20. Ames PR, Tommasino C, Iannaccone L, et al.: Coagulation activation and fibrinolytic imbalance in subjects with idiopathic antiphospholipid antibodies—a crucial role for acquired free protein S deficiency. Thromb Haemost 1996, 76:190–194.
21. Atsumi T, Khamashta MA, Andujar C, et al.: Elevated plasma lipoprotein (a) level and its association with impaired fibrinolysis in patients with antiphospholipid syndrome. J Rheumatol 1998, 25:69–73.
22. Li XN, Grenett HE, Benza RL, et al.: Genotype-specific transcriptional regulation of PAI-1 expression by hypertriglyceridemic VLDL and Lp(a) in cultured human endothelial cells. Arterioscler Thromb Vasc Biol 1997, 17:3215–3223.
23. •• Chen PP, Yang CD, Ede K, et al.: Some antiphospholipid antibodies bind to hemostasis and fibrinolysis proteases and promote thrombosis. Lupus 2008, 17:916–921. Although the major autoantigen of APS is considered to be β_2GPI, this article demonstrates that some aPL are capable of binding to homologous domains of key proteases involved in hemostasis and fibrinolysis.
24. Yang CD, Hwang KK, Yan W, et al.: Identification of anti-plasmin antibodies in the antiphospholipid syndrome that inhibit degradation of fibrin. J Immunol 2004, 172:5765–5773.
25. Lu CS, Horizon AA, Hwang KK, et al.: Identification of polyclonal and monoclonal antibodies against tissue plasminogen activator in the antiphospholipid syndrome. Arthritis Rheum 2005, 52:4018–4027.
26. Cugno M, Cabibbe M, Galli M, et al.: Antibodies to tissue-type plasminogen activator (t-PA) in patients with antiphospholipid syndrome: evidence of interaction between the antibodies and the catalytic domain of t-PA in 2 patients. Blood 2004, 103:2121–2126.
27. Lutters BC, de Groot PG, Derksen RH: beta 2 Glycoprotein I—a key player in the antiphospholipid syndrome. Isr Med Assoc J 2002, 4:958–962.
28. Valesini G, Alessandri C: New facet of antiphospholipid antibodies. Ann N Y Acad Sci 2005, 1051:487–497
29. •• D'Ippolito S, Di Simone N, Di Nicuolo F, et al.: Antiphospholipid antibodies: effects on trophoblast and endothelial cells. Am J Reprod Immunol 2007, 58:150–158. This article illustrates a possible mechanism of how aPL can cause pregnancy complications and miscarriage in APS patients.
30. Kamboh MI, Mehdi H: Genetics of apolipoprotein H (fl2-glycoprotein I) and anionic phospholipid binding. Lupus 1998, 7:S10–S13.
31. Horbach DA, van Oort E, Lisman T, et al.: Beta2-glycoprotein I is proteolytically cleaved in vivo upon activation of fibrinolysis. Thromb Haemost 1999, 81:87–95.
32. Yasuda, S, Atsumi, T, Ieko, M, et al.: Nicked β2-glycoprotein I: a marker of cerebral infarct and a novel role in the negative feedback pathway of extrinsic fibrinolysis. Blood 2004, 103:3766–3772.
33. López-Lira F, Rosales-León L, Martínez VM, et al.: The role of beta2-glycoprotein I (beta2GPI) in the activation of plasminogen. Biochim Biophys Acta 2006, 1764:815–823.
34. •• Bu C, Gao L, Xie W, et al.: beta2-glycoprotein i is a cofactor for tissue plasminogen activator-mediated plasminogen activation. Arthritis Rheum 2009, 60:559–568. This article presents a role for β_2GPI in the regulation of fibrinolysis by showing that β_2GPI interacts with tPA and induces tPA-dependent plasminogen activation in the fluid phase and that this process is inhibited by antibodies to β_2GPI.
35. Lázaro I, Carmona F, Reverter JC, et al.: Antiphospholipid antibodies may impair factor XIIa–dependent activation of fibrinolysis in pregnancy: in vitro evidence with human endothelial cells in culture and monoclonal anticardiolipin antibodies. Am J Obstet Gynecol 2009, 201:e1–e6.
36. Ma K, Simantov R, Zhang JC, et al.: High affinity binding of beta 2-glycoprotein I to human endothelial cells is mediated by annexin II. J Biol Chem 2000, 275:15541–15548.
37. Hajjar KA, Jacovina AT, Chacko J: An endothelial cell receptor for plasminogen/tissue plasminogen activator. I. Identity with annexin II. J Biol Chem 1994, 269:21191–21197.
38. Cesarman GM, Guevara CA, Hajjar KA: An endothelial cell receptor for plasminogen/tissue plasminogen activator (t-PA). II. Annexin II-mediated enhancement of t-PA-dependent plasminogen activation. J Biol Chem 1994, 269:21198–21203.
39. Cesarman-Maus G, Ríos-Luna NP, Deora AB, et al.: Autoantibodies against the fibrinolytic receptor annexin 2, in antiphospholipid syndrome. Blood 2006, 107:4375–4382.