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Review Article

Receptors involved in cell activation by antiphospholipid antibodies

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ABSTRACT

The antiphospholipid syndrome (APS) is an autoimmune disease associated with arterial or venous thrombosis and/or recurrent fetal loss and is caused by pathogenic antiphospholipid antibodies (aPLA). The plasma protein β2-glycoprotein 1 (β2GP1) has been identified as a major target of aPLA associated with APS. Cell activation by aPLA appears to be a major pathogenic cause in the pathogenesis of APS. Receptors, co-receptors and accessory molecules are known to assist the pathogenic effects of aPLA. Members of the TLR family and the platelet receptor apolipoprotein E receptor 2' (apoER2'), a receptor belonging to the low-density lipoprotein receptor (LDL-R) family, as well as GPIbα, were identified as putative candidates for aPLA recognition. CD14, a co-receptor for TLR2 and TLR4, and annexin A2, a ubiquitous Ca2+-binding protein that is essential for actin-dependent vesicle transport, could serve as important accessory molecules in mediating the pathogenic effects of aPLA. Finally, complement activation has been reported in association with the pathogenicity of APS. The relative contribution of these different mechanisms in the pathogenesis of APS is controversial. Here, we review the various *in vivo* and *in vitro* models that have been used to investigate the pathogenic mechanisms of aPLA in APS.

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Contents

Introduction

The antiphospholipid syndrome (APS) is characterized by the presence of antiphospholipid antibodies (aPLA) and clinical manifestations such as recurrent thromboembolic events and/or pregnancy complications [1]. APS may be concomitant with systemic lupus erythematosus (SLE), other autoimmune diseases, malignant diseases and bacterial or viral infections (secondary APS), but may also occur without an underlying disease (primary APS). The diagnosis of APS is complicated by the polyclonal nature of the autoantibodies and the variety of target antigens. Moreover, heterogeneous antibodies may co-exist in the same patient [2]. The main antigenic target of aPLA is the plasma phospholipid binding protein β2-glycoprotein 1 (β2GP1). Studies on the predictive pathogenic value of the different types of aPLA seem to indicate that antibodies with biological lupus anticoagulant activity (LA) as well as antibodies against β2GP1 or anticardiolipin (aCL) correlate with a history of thrombo-embolic complications and pregnancy morbidity. Patients that are triple positive for LA, anti-β2GP1 and aCL antibodies have the highest risk of complication [3,4].

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Patients with persistent aPLA present only occasional thrombotic episodes, and, sometimes, bacterial or viral infections are associated with the occurrence of clinical manifestations [5]. These observations underline that aPLA alone are not sufficient to induce thrombosis formation. Most likely, a «priming factor» from infectious or inflammatory origins is needed. The implication of infection seems particularly obvious in catastrophic APS, a rare but often fatal subset of APS, which presents some characteristic features comparable to those occurring in septic shock [5].

Several mechanisms have been proposed to explain the pathogenic effects of aPLA. These may be divided in two non-mutually exclusive broad mechanisms: 1) the inhibition of anticoagulant pathways dependent on protein C, antithrombin or annexin V or of fibrinolysis [6–8] or 2) the pro-inflammatory, pro-coagulant activation of endothelial cells, monocytes or platelets [9].

Here we describe the mechanisms by which aPLA are able to induce a pro-inflammatory and pro-coagulant state into monocytes, endothelial cells (EC) and platelets. We will review the role of β2GP1 as a major target of cell activating antibodies, the importance of receptors, co-receptors and accessory proteins in mediating cell activation, and finally the contribution of receptor internalization to cell activation.

Cell activation by aPLA

A large number of studies have shown that aPLA are able to induce inflammatory activation of EC, monocytes and platelets [5,10,11]. Experimental studies in mice suggest that EC stimulation by aPLA play an important role in APS. Immunization of mice with β2GP1 or injection of human aPLA in mice increased thrombus size in murine thrombosis models and induced recurrent pregnancy loss. Thrombus formation was significantly reduced or abrogated in mice lacking the leukocyte adhesion molecules ICAM-1, E-selectin or P-selectin or by injection with anti-VCAM-1 antibodies [12,13]. These results imply that activation of the vascular endothelium by aPLA is a major thrombogenic mechanism [14]. Several *in vitro* as well as *in vivo* studies have shown that aPLA may recognize target antigens, such as β2GP1, bound to the surface of EC [15]. The specific binding of anti-β2GP1 antibodies to EC induces surface expression of pro-coagulant, pro-adhesive and pro-inflammatory molecules that favors the binding of circulating leukocytes. Activated EC upregulate expression of tissue factor (TF), leukocyte adhesion molecules (VCAM-1, ICAM-1 and E-selectin), inflammatory cytokines/chemokines (IL-6, fractalkine and MCP-1), and the fibrinolysis inhibitor PAI-1. Activation also leads to decrease expression of thrombomodulin, a cofactor of the activated protein C anticoagulant pathway. These different changes facilitate the adhesion of leukocytes that become activated and express TF and thereby further enhance local procoagulant activity increasing the risk of thrombotic occlusions [11,16,17]. Consistent with this view, circulating monocytes expressing TF have been observed in patients with primary APS associated with thrombotic events [18].

Mice deficient for the complement factors C3 and C5 are resistant to thrombosis or recurrent pregnancy loss induced by aPLA. These observations suggest that activation of the complement cascade contributes to the pathogenic effects of aPLA, most likely via the capacity of C5a in placental tissues to attract and activate immune cells and to stimulate the release of inflammatory mediators, including TNF-α [19–21].

The various complications observed during pregnancy of APS patients have been associated with mechanisms partly separate from thrombosis, inflammation or activation of complement. Data indicate that aPLA may reduce decidual and vascular trophoblast invasion, induce extravillous and syncytiotrophoblast activation, disrupt syncytium formation and promote thromboembolism in decidual vessels leading to preeclampsia [22,23].

The main antigenic target for aPLA: β2GP1

Although it was initially thought that aPLA bound directly to anionic phospholipids, it is now commonly accepted that aPLA directed against a plasma protein with affinity for anionic phospholipids, namely β2GP1, are clinically important [11]. β2GP1 is composed of five "sushi" domains. Domain V mediates the binding of the molecule to anionic phospholipids while domain I seems to be the main target of antibodies associated with an increased risk of thrombosis [24,25]. At least two different conformations are known for β2GP1: a circular plasma conformation in which domain I interacts with domain V and an "activated" fishhook-like conformation [26]. The fishhook-like conformation is obtained after binding of positively charged patch of domain V to anionic phospholipids. The dissociation of domain I and domain V leads to the exposure of an epitope containing the amino acids Arg39 and Arg43 that are critical for binding of pathogenic aPLA [26]. While the contribution of anti-β2GP1 antibodies in APS is well described, the physiological function of β2GP1 remains to be established. Indeed, human homozygotes for β2GP1 deficiency do not present any clinical complications [27]. The function of β2GP1 proteins in APS was recently re-evaluated by Agar et al. [28]. β2GP1 appears to modulate the innate immune system through its ability to downregulate LPS-induced TF and IL-6 expression by monocytes. The interactions of LPS with domain V of β2GP1 lead to conversion of the circular conformation to the fishhook-like conformation. These results are consistent with the "two hit hypothesis". Indeed, primary infection could provide the first hit leading to the fishhook-like conformation that exposes the epitope recognized by aPLA. Binding of aPLA then induces pro-inflammatory and pro-coagulant activation of monocytes and EC. In addition, the first hit could also lead to the expression of aPLA/β2GP1 plasma membrane associated receptors that are not constitutively present (see below).

Candidate receptors for aPLA

Several candidate receptors have been proposed for aPLA: *in vivo* studies have shown that deficiencies of TLR4 [29], Annexin A2 [30], ApoER2 [31] and several complement factors [20] reduced the pathogenic effects of aPLA. Moreover, *ex vivo* studies suggest a role for TLR2 [32–34], CD14 [34], GPIbα [35] and TLR8 [36] in aPLA-activated monocytes and EC. Below the role of each receptor/co receptor is described in more detail.

The role of members of the TLR family

TLRs belong to type I membrane proteins family that recognize conserved pathogen-associated molecular patterns (PAMPs). These receptors thereby function as the first line of defense against pathogens and are essential factors in the innate immune response [37]. TLRs are expressed on immune cells, epithelial cells, EC and platelets. They are implicated in the pathogenesis of autoimmune diseases such as rheumatoid arthritis and lupus erythematous [38]. The formation of TLR complexes with co-receptors affords the generation of a combinatorial repertoire to discriminate among diverse signals. Activated TLR receptor complexes then recruit intracellular effector proteins. Of importance, TLR4 recognizes lipolysaccharide (LPS) and TLR2 recognizes bacterial lipopeptides. TLR4/MD2 forms homodimers, whereas TLR2 heterodimerizes with TLR1 or TLR6 [39,40]. In this system, CD14 is a co-receptor for both TLR4 and TLR2 whereas the scavenger receptor CD36 and the integrin CD11a participate exclusively to TLR2 activities [41,42]. TLRs may also depend on other co-receptors for full ligand sensitivity. Upon stimulation, TLR complexes are internalized and targeted to endosomal compartments [42–45]. Receptor internalization may also serve as an important endogenous mechanism that protects against chronic inflammation by avoiding unrestrained signaling by TLRs [46]. While some studies have reported that TLR4 is essential for the pathogenic effects of aPLA, other studies underline the central role of TLR2. The relative importance of TLR4 and TLR2 in APS remains controversial.

We address here the possible reasons for these contradictions, addressing the models used in past studies. Human umbilical vein EC (HUVEC) express high levels of TLR4 and low amounts of TLR1, TLR2, and TLR6 [47,48]. Increased expression of TLR2 by EC in response to inflammatory mediators such as TNF, LPS, IL-1β, IFNγ and histamine *in vivo* and *in vitro* [49–52], however, provides a mechanism allowing EC to respond to TLR2 ligands under inflammatory conditions. TLR2 and TLR4 are both expressed on platelets [53] and on monocytes [54]. Sorice et al. [55] have identified that TLR4, β2GP1 and annexin A2 form an activation cluster in plasma membrane microdomains of human monocytes after LPS or aPLA ligation. This study however did not evaluate the association of β2GP1 with other TLRs in lipid rafts in monocytic cells, and in particular because TLR2 and CD14 are required for aPLA recognition by monocytes and EC [34]. By using the proximity ligation assay, a technique that generates information concerning the strength of the protein-protein interactions, aPLA were found to interact more strongly with TLR2 on monocytes and cytokine activated EC than with TLR4 [34]. The pathogenesis of APS is further complicated by the more recent findings that show that TNF production induced by aPLA-activated monocytes could be dependent on activation of TLR7 and TLR8 [36,56,57]. Further study is thus required to reach definitive conclusions.

The "two-hit hypothesis" postulates that although the persistence of elevated levels of antibodies directed against of β2GP1 is a necessary condition, the occurrence of APS is seemingly triggered by an additional "second hit". However, we could consider that inflammatory responses may represent the "first hit" whereas pathogenetic antibodies against anti-β2GP1 act as the "second hit". This point of view is supported the study of Fischetti et al. since rats administered aPLA with anti-β2GPI activity have spontaneous thrombosis if they also receive LPS before IgG infusion [58]. These findings also raise the possibility that infectious agents such as bacteria may play a dual role in APS pathogenesis [59]. They may elicit an inflammatory response that serves as the first hit, and then by triggering the generation of cross-reactive anti-β2GPI antibodies (second hit) due to sequence homology between target epitopes for anti-β2GPI antibodies and a number of common pathogens [60]. While the nature of possible first-hit conditions is still elusive, accumulating evidence, although controversial, suggest that the responses of EC, monocytes or platelets to aPLA require interactions with innate immune receptors, co-receptors and accessory proteins. As discussed above, whereas under resting conditions EC abundantly express TLR4 molecules, but little TLR2 proteins, EC activation by TNF or TLR4 ligands increases TLR2 levels [52] and aPLA-associated response of EC [34]. While the exact role of TLR4 or TLR2 in EC activation by aPLA remains to be defined, and may likely depend on the relative expression of these TLRs by EC, we propose that a first hit, such as an infectious insult, induces TLR2 up-regulation by EC through a TLR4-dependent mechanism. In addition, LPS or other bacterial PAMPs may induce the conformational change of β2GP1 into the fishhook-like structure, which facilitates the interaction of aPLA with β2GP1, thereby increasing their pathogenic effects. In case of pregnancy morbidity, instead of infection, hormonal changes could play a role in aPLA pathogenicity [61,62].

ApoER2'and GPIbα as aPLA receptors

As mentioned, the low-density lipoprotein (LDL) receptor-related protein-8 (LRP8; also known as ApoER2') and GPIbα appear also to play an important role in mediating the pathogenic effects of aPLA [63,64]. The interaction of dimerized β2GP1 with either ApoER2' or GPIbα was found to lower the threshold of platelet activation by collagen or fibronectin surfaces [31,65]. While EC and monocytes do not
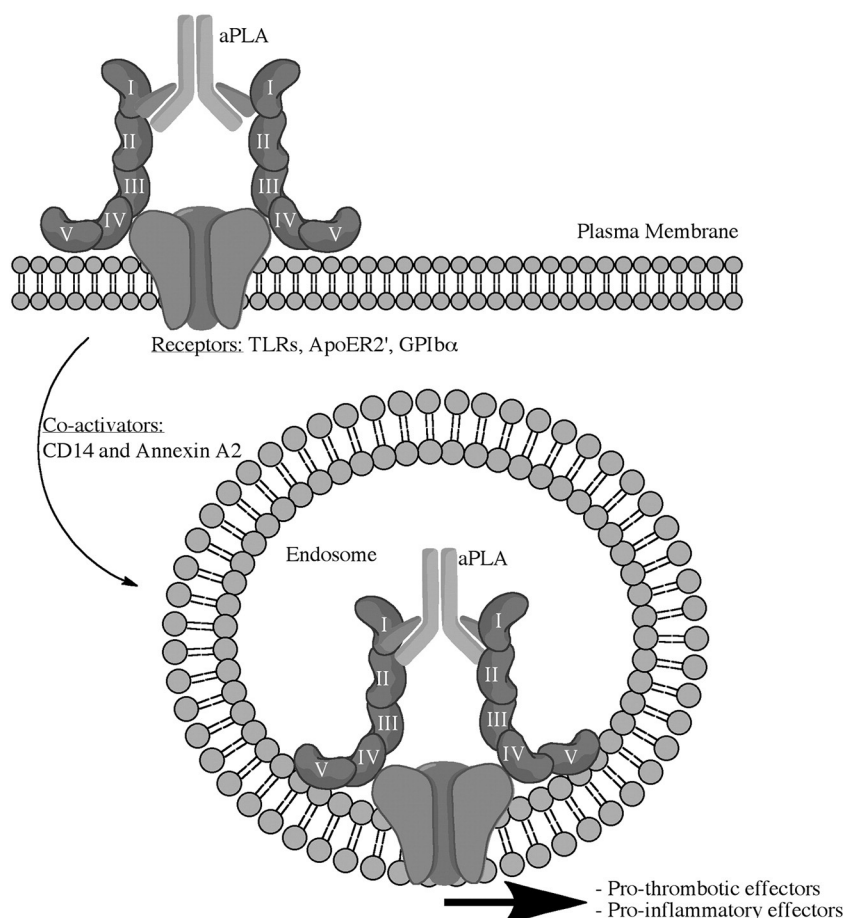

Fig. 1. Schematic representation of aPLA/β2GP1 internalization with the participation of potential receptors namely TLRs, ApoER2', GPIbα and co-activators (accessory molecules) such as Annexin A2 or CD14.

Table 1
Candidate of aPLA/β2GP1 receptors and co-activators: Modulation of expression by inflammatory stimuli or other factors and implication in endocytosis.

NAME	MODULATION OF EXPRESSION	ENDOCYTOSIS	CELLS TYPES OR TISSUES	REFERENCES
RECEPTORS				
TLR2	Yes	Yes	EC, MΦ, Placenta, Platelets	[45,52]
TLR4	Yes	Yes	EC, MΦ, Placenta, Platelets	[75,88]
TLR7/8	Yes	Yes	Dendritic cells, MΦ	[56,89]
ApoER2'	Yes	Yes	MΦ, Placenta, Platelets	[31,90]
GPIbα	Yes	Yes	Platelets	[91,92]
CO-ACTIVATORS				
CD14	Yes	Yes	EC, MΦ, Placenta, Platelets	[44,93]
Annexin A2	Yes	Yes	EC, MΦ, Placenta	[72,94]

EC: Endothelial cell; MΦ: Monocytes/macrophages.

express ApoER2', other members of LDL-receptor family were shown to bind the dimerized form of β2GP1 [64,66]. Further studies are needed to clarify the importance and mechanism by which GPIbα and LDL-receptor family of proteins may contribute to aPLA-mediated activation of target cell in APS.

Annexin A2 as an accessory protein for aPLA

In addition to CD14 previously mentioned, participation of accessory proteins such as annexin A2 in aPLA-mediated EC activation has been suggested. Annexin A2 is a Ca^{2+}-dependent phospholipid-binding protein known to associate with the plasma membrane and the endosomal system and to play an important role in endocytosis and exocytosis. Interestingly, annexin A2 was found to mediate high affinity binding of β2GP1 to unstimulated EC [67]. Anti-annexin A2 antibodies as well as bi-valent anti-annexin A2 (Fab')₂ fragments were able to activate EC whereas monomeric Fab fragments prevented the activation induced by anti-annexin A2 or anti-β2GP1 antibodies [68]. Furthermore, anti-annexin A2 antibodies isolated from plasma of patients are equally efficient as anti-β2GP1 antibodies to induce TF expression by EC [69]. Anti-annexin A2 antibodies increased also thrombosis extent in a mouse model of thrombosis [30]. These results suggest that EC activation trigger by anti-β2GP1 or anti-annexin A2 antibodies induce a cross-linking or clustering of annexin A2/β2GP1 complexes at the cell surface sufficient to trigger EC activation. Whereas annexin A2 is not a transmembrane protein, interaction of annexin 2 or β2GP1 with transmembrane receptors is likely necessary to transduce intracellular signaling.

Internalization and aPLA receptors

Although not yet addressed, the contribution of multiple candidate signaling receptor in aPLA-mediated cell activation may potentially reflect the process endorsing internalization of aPLA, a mechanism by which aPLA could contribute to several of the proposed pathogenic mechanisms leading to APS. In particular, we have previously suggested that the translocation of aPLA into late endosomes may contribute to their pathogenic effects [15,57,70]. Interestingly, annexin A2 appears to be a necessary component of the machinery controlling endosomal membrane dynamics and multivesicular endosome biogenesis, and, in particular the clathrin-dependent endocytosis [71]. Annexin A2 also participates in the regulation of actin dynamics which is an important step in the endocytosis machinery [72]. ApoER2' may also facilitate endocytosis and signal transduction [73]. While the internalization of TLR4 is required for optimal activity in response to a wide variety of ligands [74,75], our data also suggest that TLR2 endocytosis in monocytes also provides an optimal mechanism for monocyte activation by TLR2 ligands, including aPLA (Brandt et al., submitted). Adding further to the complexity regarding the interaction of TLRs with their respective ligands is the observation that specific TLRs may interact with co-receptors of other TLRs. Spitzer et al. [76] have demonstrated that TLR1 interacts and modulates the signaling of TLR4 and also that CD14 is required for both TLR4- and TLR2-dependent signaling. Finally, CD14 also interacts with endosomal TLR8 [77–79]. So

many candidate receptors of aPLA seem to be involved in endocytosis process suggesting a commonality in aPLA-mediated cell activation (Fig. 1 and Table 1). Thus, additional studies aimed at understanding the relation between endocytosis and potential aPLA receptors are necessary to resolve the apparent contradiction.

Signal transduction pathways mediating cell activation by aPLA

Different signal transduction pathways have been identified to mediate the activation of monocytes and/or EC in response to aPLA. Among the important signaling intermediates that play a central role in this process are proximal mediators of innate responses, such as NF-kB [80–82], p38-MAP kinase [83,84], ERK [85] and IRAK [55] as well as TRIF, MyD88 and TRAF6 [86]. In addition, the PI3K/Akt pathway seems to play a specific role in platelet activation by aPLA. Indeed, treatment of platelets with antibodies against β2GP1 induces the phosphorylation of Akt [87]. To date, the characterization of the signaling pathways induced by aPLA does not allow the identification of the key receptors in aPLA response, as these various intracellular transduction pathways are common to many receptors.

Conclusion

Antiphospholipid antibodies-mediated cell activation appears to be an important pathogenic mechanism associated with APS. Currently, there is no consensus as to which receptors, co-receptors and accessory proteins are mainly responsible for cell activation by aPLA. Further comparative *in vivo* and *in vitro* studies are therefore required to clarify the respective or synergistic roles of the various receptors documented playing a role in APS, particularly with a focus on the importance of endocytosis of aPLA.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgements

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