Published in final edited form as:

Lupus. 2008 October ; 17(10): 943–951. doi:10.1177/0961203308095329.

Annexin A2: biology and relevance to the antiphospholipid syndrome

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Abstract

Antiphospholipid antibodies (aPL), the majority of which are directed against β_2 -glycoprotein I (β_2 GPI), are associated with an increased incidence of venous and arterial thrombosis. The pathogenesis of antiphospholipid/anti- β_2 GPI-associated thrombosis has not been defined, and is likely multifactorial. However, accumulating evidence suggests an important role for endothelial cell activation with the acquisition of a procoagulant phenotype by the activated endothelial cell. Previous work demonstrated that endothelial activation by antiphospholipid/anti- β_2 GPI antibodies is β_2 GPI-dependent. We extended these observations by defining annexin A2 as an endothelial β_2 GPI binding site. We also observed that annexin A2 plays a critical role in endothelial cell activation induced by anti- β_2 GPI antibodies, and others have described direct endothelial activation by anti-annexin A2 antibodies in patients with aPL. Similar findings have been reported using human monocytes, which also express annexin A2. Because annexin A2 is not a transmembrane protein, how binding of β_2 GPI/anti- β_2 GPI antibodies, or anti-annexin A2 antibodies, to endothelial annexin A2 causes cellular activation is unknown. Recent studies, however, suggest an important role for the Toll-like receptor family, particularly TLR4. In this article, we review the role of these interactions in the activation of endothelial cells by aPL. The influence of these antibodies on the ability of annexin A2 to enhance t-PA-mediated plasminogen activation is also discussed.

Keywords

annexin; antiphospholipid; β_2 -glycoprotein I; endothelial; thrombosis

Introduction

The antiphospholipid syndrome (APS) is among the most important of the acquired thrombophilic states. This disorder is associated with an increased risk of primary arterial and venous thrombosis, and its persistence after an initial vascular event increases the risk for recurrent thrombosis and cardiovascular mortality.¹ Despite the clinical significance of the APS, its overall pathogenesis is not well understood.² Pathogenic mechanisms may vary among patients, and it is likely that in most affected individuals the pathogenesis of thrombosis is multifactorial.

Endothelial cells play an essential role in maintaining blood fluidity through the expression of naturally occurring anticoagulants on the endothelial cell surface.^{3,4} Disruption or activation of endothelial cells leads to loss of the anticoagulant phenotype and assumption of pro-inflammatory, prothrombotic properties.⁴⁻⁶ Many reports have shown that antiphospholipid antibodies (aPL) may activate endothelial cells *in vitro*^{2,7-9} and this appears to occur *in vivo* as well.¹⁰ These considerations support the hypothesis that endothelial cell activation plays a prominent role in the pathogenesis of the APS. Moreover, because many of the reported pathogenic activities of aPL may occur indirectly as a consequence of endothelial cell activation, activation of endothelial cells may be a primary event in the pathophysiological cascade leading to thrombus development in patients with APS.

We have previously reported that annexin A2, a member of a large family of annexin proteins, serves as an endothelial cell binding site for the antiphospholipid cofactor, β_2 -glycoprotein I (β_2 GPI).¹¹ Additional studies suggest that binding of β_2 GPI to endothelial cell annexin A2 plays a critical role in subsequent activation of endothelial cells by anti- β_2 GPI antibodies.⁷ Moreover, anti-annexin A2 antibodies that are capable of directly activating endothelial cells also occur in patients with APS.¹² Human monocytes also express annexin A2, and their activation may also contribute to the pathogenesis of the APS. In this report, we will provide a brief review of the biology of annexin A2, and discuss its potential role in antiphospholipid/anti- β_2 GPI-antibody-mediated thrombosis.

Annexin A2

Overview

The annexins comprise a large family of structurally related proteins, which share the ability to bind to negatively charged phospholipids in a Ca^{2+} -dependent manner.¹³ This binding is reversible, and chelation of Ca^{2+} liberates annexins from the phospholipid matrix. Each member of the annexin family is composed of two principal domains: a unique amino-terminal “tail” domain, and a conserved COOH-terminal protein core¹³ (Figure 1). The latter, which consists of a series of 70 amino acid ‘endonexin’ repeats, shares approximately 40–70% homology among annexin family members and mediates the binding of annexins to phospholipid and cell membranes.¹⁴ This domain also contains F-actin binding and bundling sites, as well as heparin binding sites.¹⁵ Unlike the core domain, the length and amino acid composition of the tail domain is highly variable among different annexins.¹⁴ Unlike most annexins, and despite the lack of a hydrophobic signal peptide, the presence of annexin A2 on cell surfaces is well established;¹⁴ approximately 4.3% of total endothelial annexin A2 is associated with the external plasma membrane.¹⁶ Association of annexin A2 with the cell surface is thought to be mediated by a KGLGT sequence (residues 118–122) and a coordinating D161 residue that reside within endonexin core repeat 2.^{13,16}

Annexin A2 mediates a number of activities that regulate several cellular functions. For example, annexin A2 binds F-actin and mediates Ca^{2+} -dependent filament bundling.^{13,18} These effects may play an important role in the organization of membrane-associated actin at sites of cholesterol-rich membrane domains (lipid rafts), in which annexin A2 may be concentrated.¹⁹ Indeed, in the presence of Ca^{2+} , annexin A2 binds to and may promote the lateral association as well as the organization of such glycosphingolipid and cholesterol-rich lipid microdomains,^{20,21} a property that may require association of annexin A2 with the actin cytoskeleton.¹³ This property may also underlie the potential contributions of annexin A2 to endo and exocytotic pathways.²²

Annexin A2 mediates the binding of β_2 -glycoprotein I (β_2 GPI) to endothelial cells

Most pathogenic aPL, detected either by their ability to prolong phospholipid-dependent anticoagulation tests (lupus anticoagulants; LAC) or their binding to cardiolipin-coated wells (anticardiolipin antibodies: ACA), are directed against β_2 GPI.^{2,23–26} A subset of patients with clinical characteristics of the APS but negative tests for LAC or ACA may have anti- β_2 GPI antibodies detected only by a direct anti- β_2 GPI ELISA.^{27,28} Such antibodies have recently been incorporated into the laboratory criteria for the APS.¹

β_2 GPI is an abundant plasma glycoprotein, first described by Schulze, *et al.* in 1961,²⁹ that consists of five homologous 60-amino acid domains.³⁰ The first four of these are classical short consensus repeat domains with extensive homology to those found in the complement-type repeats of Factor H.³¹ Each of these contains four conserved cysteines with a characteristic C_{1–3}, C_{2–4} disulfide bonding pattern, whereas domain 5 contains six cysteines, with C_{1–4}, C_{2–5} and C_{3–6} disulfide linkages.^{32–34} Domain 5 is also unique in its high content of lysine residues^{35,36} that mediate the binding of β_2 GPI to anionic phospholipids.^{36,37} Dimerization of β_2 GPI, which may be induced by bivalent anti- β_2 GPI antibodies, dramatically enhances its phospholipid binding activity.³⁸

Previous studies from our laboratory focused on the interactions of β_2 GPI with endothelial cells, and led to the identification of annexin A2 as a high affinity endothelial cell surface β_2 GPI binding protein.¹¹ We reported that ¹²⁵I- β_2 GPI bound with high affinity ($K_d \sim 18$ nM) to unstimulated human umbilical vein endothelial cells (HUVEC), and subsequently used an affinity purification strategy, with β_2 GPI as the immobilized ligand, to isolate β_2 GPI binding proteins of approximately 36 and 78 kDa from HUVEC. Analysis of tryptic digests of these proteins by LC-MS showed peptide sequences exclusively corresponding to those within annexin A2. A role for annexin A2 in binding of β_2 GPI to cells was confirmed in subsequent studies, in which we reported that 1) binding of β_2 GPI to endothelial cells was blocked by monoclonal and polyclonal anti-annexin A2 antibodies, 2) transfection of HEK-293 cells with annexin A2 cDNA led to a 10-fold increase in binding of ¹²⁵I- β_2 GPI and 3) high affinity binding of β_2 GPI and annexin A2 to each other also occurred in cell free systems, including ELISA and surface plasmon resonance assays.¹¹

The role of β_2 GPI-annexin A2 interactions in endothelial cell activation

Activation of endothelial cells may play a central role in the development of thrombosis in patients with APS,^{2,38–42} and several reports have showed that plasma from patients with the APS contains antibodies that bind and activate endothelial cells.^{8–10,43,44} Moreover, endothelial cell activation by such antibodies was shown by Simantov, *et al.*⁸ to occur in a β_2 GPI-dependent manner. The possible clinical relevance of endothelial cell activation in patients with APS is supported by reports showing increased plasma levels of tissue factor⁴⁵ and VCAM-1,⁴⁶ as well as endothelial microparticles,^{47,48} in these individuals.

Previous studies performed in our laboratory have demonstrated that antiphospholipid/anti- β_2 GPI antibodies activate endothelial cells through cross-linking or clustering of annexin A2-bound β_2 GPI on the endothelial surface.⁷ This conclusion is based on several observations. First, both intact anti-annexin A2 antibodies and anti-annexin A2-derived F(ab')₂ fragments (bivalent) induced endothelial cell activation, whereas anti-annexin A2 Fab fragments (monovalent) did not. Second, the magnitude and time course of endothelial cell activation induced by anti-annexin A2 antibodies or anti- β_2 GPI antibodies in the presence of β_2 GPI was indistinguishable. Third, anti-annexin A2 antibody-derived Fab fragments (monomeric) blocked endothelial cell activation caused by intact anti-annexin A2 antibodies, as well as that caused by anti- β_2 GPI antibodies in the presence of β_2 GPI. These results suggest that annexin A2 clustering induced by either bivalent anti-annexin A2

antibodies or F(ab')₂ fragments, or bivalent anti-β₂GPI antibodies in the presence of annexin A2-bound β₂GPI, induces endothelial cell signalling responses that lead to endothelial cell activation and expression of the endothelial cell adhesion molecules E-selectin, VCAM-1 and ICAM-1.⁷

These observations are supported by a subsequent report from Cesarman-Maus, *et al.*¹² that described anti-annexin A2-specific antibodies in patients with the APS, which also induced endothelial cell activation. In this study, serum samples from 434 individuals (206 patients with lupus, 62 with APS, 21 with non-autoimmune thrombosis and 145 normal controls) were evaluated for the presence of anti-annexin A2 antibodies by ELISA and/or immunoblotting. Anti-annexin A2 antibodies were detected in three of the 145 normal controls (2.1%) and in 6.3% of patients with systemic lupus who had not had thrombosis. Anti-annexin A2 antibodies were not detected in any patients with thrombosis associated with hereditary thrombophilia or acquired non-immune risk factors. On the contrary, anti-annexin A2 antibodies (>3 SD) were observed in 22.6% of patients with APS, including 17.5% of patients with venous, 29.4% of patients with arterial and 40% of patients with mixed thrombosis. Antibodies occurred with equal frequency in patients with primary and secondary APS, but were significantly more common (25.8%) in patients with lupus-associated APS than in patients with lupus who had not experienced thrombosis (6.3%). Anti-annexin A2 IgG from these patients induced endothelial cell activation, enhancing the expression of endothelial cell tissue factor by 6.4-fold.¹²

How does annexin A2 clustering induce endothelial cell activation?

The mechanisms by which cross-linking of annexin A2-bound β₂GPI by anti-β₂GPI antibodies, or direct cross-linking of annexin A2 by anti-annexin A2 antibodies induces endothelial cell activation has not been clearly defined. Although annexin A2 may be phosphorylated by pp60^{c-src} in response to membrane binding,⁴⁹ and has been implicated in insulin signal transduction,⁵⁰ it is not a transmembrane protein and the mechanisms by which it might induce outside-in signalling events in response to binding of β₂GPI and anti-β₂GPI antibodies is not obvious. In previous studies, we postulated that a transmembrane 'adaptor' protein that associates laterally with annexin A2 in the cell membrane might facilitate transmembrane signal transduction.¹¹ Although this hypothesis has been received with some skepticism, accumulating evidence supports the involvement of additional cellular protein(s) in mediating endothelial cell activation in response to anti-β₂GPI antibodies. However, the exact mechanisms by which β₂GPI and anti-β₂GPI antibodies activate endothelial cells have yet to be fully delineated.

Clues to potentially relevant signalling pathways are provided by consideration of current knowledge concerning signalling pathways responsible for endothelial activation, and reports that have examined endothelial cell activation in response to anti-β₂GPI antibodies. As noted previously, endothelial cells activated by anti-β₂GPI antibodies in the presence of β₂GPI, or directly by anti-annexin A2 antibodies, show increased cell surface expression of adhesion molecules, including ICAM-1, VCAM-1 and E-selectin,^{7,8} as well as tissue factor. Similar responses that occur in response to inflammatory cytokines such as tumour necrosis factor alpha (TNFα) depend upon NFκB-dependent gene transcription, which occurs following release of NFκB from IκB and its translocation to the nucleus.⁵¹⁻⁵⁴ Dunoyer-Geindre, *et al.*⁵⁵ were the first to show that incubation of endothelial cells with anti-β₂GPI antibodies led to nuclear translocation of NFκB that was linked to expression of endothelial cell tissue factor, ICAM-1, VCAM-1 and E-selectin. These investigators also reported that BAY11-7085, which inhibits NFκB nuclear translocation, blocked the increased expression of adhesion molecules caused by anti-β₂GPI antibodies, confirming a central role for NFκB in the endothelial cell activation response to these antibodies.⁵⁵

An important outside-in signalling pathway that culminates in NF κ B activation is that triggered by ligand binding through toll-like receptor (TLR) family members, particularly TLR4.^{56–59} The cytoplasmic domains of this family of transmembrane ‘pattern recognition’ receptors displays extensive homology to those of the interleukin-1 receptor (IL-1R) family, thus leading to their designation as Toll/IL-1R (TIR) domains.⁵⁶ At least 10 distinct TLR family members have been identified in humans,^{56,57} with TLR-4 serving as the primary lipopolysaccharide (LPS) signalling receptor. Through CD14, LPS is transferred to the receptor complex of TLR-4 and an adaptor protein, MD2, upon which a cascade of signalling events culminating in NF κ B activation ensues (Figure 2).⁶⁰ Definition of this pathway has been aided by studies in the C3H/HeJ mouse strain, in which the *tlr4* gene contains a missense mutation in the region encoding the cytoplasmic tail (A to C substitution at position 2342), which results in the change of a highly conserved proline to histidine at position 712, and a loss of responsiveness to LPS.⁶¹ The loss of TLR4 signalling in response to LPS renders these animals resistant to LPS challenge.⁶¹

Direct evidence for a role of the TLR4 signalling pathway in activation of endothelial cells by anti- β_2 GPI antibodies was first provided by Raschi, *et al.*⁶² These investigators compared the ability of dominant negative constructs of MyD88 (myeloid differentiation protein) Traf2 (TNF receptor-associated factor 2) and Traf6 (TNF receptor-associated factor 6) to block NF κ B activation (determined using a reporter gene, ELAM-NF κ B-luciferase) in an immortalized endothelial cell line, HMEC-1, in response to IL-1, TNF α , LPS and anti- β_2 GPI antibodies. They observed that the pattern of inhibition caused by these dominant negative transfectants was similar when IL-1, LPS or anti- β_2 GPI antibodies were used as the agonist (i.e., Δ Traf6 and Δ MyD88 were effective inhibitors, whereas Δ Traf2 was not; the latter inhibited TNF α -induced activation only, because of the association of Traf2 with the TNF α receptor). These results suggested that anti- β_2 GPI antibodies use a MyD88-dependent pathway for cellular activation. Moreover, these investigators also reported that anti- β_2 GPI antibodies, IL-1 and LPS induced phosphorylation of IRAK, one of the initial kinases recruited by receptors of the IL-1/TLR superfamily. However, although the kinetics of phosphorylation caused by LPS and anti- β_2 GPI antibodies were virtually identical, IL-1 induced more rapid phosphorylation of IRAK.⁶³ Moreover, specific IL-1R antagonists did not block NF κ B activation caused by anti- β_2 GPI antibodies. Taken together, these results supported a role for TLR4, as opposed to the IL-1R, as the relevant receptor involved in anti- β_2 GPI-dependent cellular activation. This conclusion is interesting, as peptides derived from β_2 GPI share similarities in amino acid sequences and perhaps structure with common bacteria and viruses,⁶⁴ and because common microbial structures are natural ligands for the TLR family of receptors.⁶⁵ Other studies (*vide infra*) support the hypothesis that interactions between TLR4 and β_2 GPI contribute to endothelial cell activation induced by anti- β_2 GPI antibodies, although at this point it is uncertain whether β_2 GPI binds to TLR4 directly or through interactions involving other proteins, one of which may be annexin A2.

In a subsequent report, Pierangeli, *et al.*⁶⁶ assessed the potential relevance of TLR4 signalling to endothelial cell activation induced by anti- β_2 GPI antibodies *in vivo*. These investigators compared the ability of IgG fractions from two patients with APS, one patient with aPL negative systemic lupus erythematosus and one normal individual to induce leukocyte adhesion to the vasculature and thrombosis in a murine cremasteric post-capillary venule model performed in wild type and C3H/HeJ LPS^{-/-} mice (containing the TLR4 mutation described above).⁶¹ In wild-type mice, IgG fractions from patients with APS significantly increased thrombus size as well as the number of leukocytes adherent to the endothelium, although similar changes were not observed in LPS^{-/-} mice or in wild-type mice treated with β_2 GPI-depleted IgG fractions from the patients with APS. Tissue factor activity was also increased in carotid homogenates from wild-type, but not in LPS^{-/-} mice

that had been treated with the APS IgG fractions.⁶⁶ In a parallel study, the authors also determined the incidence of the Asp299Gly and Thr399Ile polymorphisms in the *tlr4* gene in 110 patients with APS. These polymorphisms, which cosegregate, have been associated with a decreased response to LPS in humans.^{67,68} In these studies, heterozygosity for the polymorphism was significantly less frequent in patients with the APS than in normal controls (4.5% vs 11%, respectively), suggesting that the wild-type allele, associated with greater TLR4 activity, might contribute to manifestations of the APS. These interesting studies, in both murine models as well as humans, suggest a potentially important role for TLR4 in the pathogenesis of the APS.

Despite these insightful studies, the mechanism by which binding of β_2 GPI to cells ultimately leads to cellular activation remains uncertain. Our studies suggest a role for annexin A2, potentially in concert with an adaptor protein such as TLR4.⁷ The studies of Raschi⁶² and Pierangeli⁶⁶ confirm the involvement of TLR4, *in vitro* and *in vivo*. However, there is little information available concerning the potential interaction between annexin A2 and TLR4, although in preliminary studies, we reported that TLR4 could be immunopurified from extracts of endothelial cells using a column of immobilized annexin A2.⁶⁹ Sorice, *et al.*⁷⁰ have examined the cellular interactions of β_2 GPI and TLR4 in more detail using isolated human monocytes, which also express abundant annexin A2. These investigators reported that annexin A2 and β_2 GPI co-immunoprecipitated in the Triton X-100 insoluble lipid raft fraction derived from these cells. Moreover, anti- β_2 GPI antibodies induced a relative shift of TLR4 from the Triton X-100-soluble to the Triton X-100-insoluble fractions. β_2 GPI could be co-immunoprecipitated from Triton X-100 insoluble fractions by anti-TLR4 antibodies, and the association between these two proteins was disrupted by methyl- β -cyclodextrin, which disrupts lipid rafts. Anti- β_2 GPI antibodies increased levels of active p65 and p50 and induced IRAK phosphorylation. Interestingly, although the MEK inhibitor PD98059 significantly inhibited anti- β_2 GPI-induced expression of tissue factor and release of TNF α , PD98059 inhibited activation of NF κ B only slightly.⁷⁰ These results suggest that these antibodies activate two distinct signalling pathways that each contribute to the activated cellular phenotype. All the β_2 GPI found in association with the Triton X-100-insoluble fractions in this study migrated at approximately 100 kD, an unusual finding attributed to β_2 GPI oxidation and dimerization. It was suggested that dimerization of β_2 GPI might induce the cellular activation state directly by inducing receptor clustering.

In summary, although the details of the pathways through which anti- β_2 GPI antibodies cause cellular activation have not been fully delineated, important roles for annexin A2 and TLR4 are suggested by evidence from several sources. Activation of the TLR4-initiated MyD88-dependent pathway as well as the MEK-1/ERK pathway may both be involved in the outside-in signalling events that lead to cellular activation. The relative importance of these pathways may vary depending on the cell type (endothelial cell, monocyte). The nature of the biochemical interactions between annexin A2, β_2 GPI, TLR4 and perhaps additional proteins have yet to be fully defined.

Does β_2 GPI affect fibrinolysis?

Annexin A2 and the annexin A2-S100A10 heterotetramer are endothelial cell receptors for plasminogen and tissue-type plasminogen activator.^{71,72} Binding of t-PA ($K_d \sim 30$ nM) and plasminogen ($K_d \sim 114$ nM) to annexin A2 enhances plasmin generation by facilitating co-assembly of these reactants, and lowering the K_M for their interaction.^{71,73} Annexin A2 mediates the binding of t-PA to endothelial cells through interactions with an LCKLSL sequence in the annexin A2 tail domain,⁷⁴ whereas plasminogen has been reported to bind annexin A2 through an internal lysine residue (K307) exposed following annexin A2 proteolysis.⁷¹ Plasminogen also binds the annexin A2:S100A10 heterotetramer via the S100A10 protein, which contains a C-terminal lysine.^{75,76} Annexin A2 enhances the

catalytic efficiency of t-PA-mediated plasminogen activation on cell surfaces by approximately 60-fold;⁷⁷ this effect is inhibited in the presence of Lys analogues or carboxypeptidase B. More potent enhancement of t-PA-mediated plasminogen activation (approximately 341-fold) is mediated by the annexin A2: S100A10 heterotetramer.^{75,76}

Several studies suggest an important physiological role for annexin A2 in fibrinolysis.⁷⁸ For example, homozygous annexin A2-null mice develop areas of fibrin deposition in the microvasculature of several organs, and display impaired clearance of injury-induced arterial thrombi.⁷⁹ Annexin A2 is abundantly expressed on the surface of human monocytes and mediates plasminogen-dependent matrix invasion by these cells,⁸⁰ and patients with acute promyelocytic leukemia, in whom annexin A2 is expressed on the surface of malignant leukemic cells, develop a hyperfibrinolytic syndrome.⁸¹ In rats, arterial thrombosis induced by oxidative injury to the vasculature caused by ferric chloride can be significantly attenuated by pretreatment with intravenous annexin A2.⁸²

The ability of annexin A2 to function as a binding site for β_2 GPI, a target for anti-annexin A2 antibodies, and a facilitator of endothelial cell surface fibrinolysis suggests that impairment of the latter activity by β_2 GPI and/or anti- β_2 GPI antibodies might result in diminished fibrinolytic activity. Indeed, Cesarman-Maus, *et al.* have reported that anti-annexin A2 antibodies isolated from patients with APS inhibit t-PA-dependent plasminogen activation in the presence of annexin A2 by 19–71%, independently of β_2 GPI (Figure 3). These antibodies also inhibit cell surface plasmin generation on endothelial cells by 34–83%.¹² However, addition of β_2 GPI to the system creates a more complex scenario, because β_2 GPI itself may enhance the activation of plasminogen. Lopez-Lira, *et al.*⁸³ have reported that β_2 GPI binds glu-plasminogen and stimulates streptokinase-mediated plasminogen activation. These investigators also reported an increase in plasmin generation on the surface of a human microvascular endothelial cell line (HMEC-1) following incubation with β_2 GPI.⁸³ These observations suggest that binding of β_2 GPI to endothelial cells may lead to enhancement of cell surface fibrinolytic activity, although whether such an effect is mediated through binding of β_2 GPI to annexin A2 has not been addressed. We have also recently observed that β_2 GPI may directly stimulate t-PA-dependent plasminogen activation, even in the absence of annexin A2, and that this activity may be inhibited by anti- β_2 GPI antibodies^a. The mechanisms by which these effects occur are currently under study.

Additional considerations suggest a role for β_2 GPI in regulation of fibrinolysis through pathways that may involve annexin A2 only indirectly. For example β_2 GPI has been reported to bind fibrin, and thus may potentially stimulate the binding of plasminogen to fibrin clots.⁸³ Yasuda, *et al.* have reported that nicked β_2 GPI, which has been cleaved by plasmin between Lys317 and Thr318 in domain 5, but not intact β_2 GPI, binds to glu-plasminogen ($K_d = 0.37 \mu\text{M}$) and suppresses plasmin generation in the presence of t-PA, plasminogen and fibrin.⁸⁴ Takeuchi, *et al.*⁸⁵ reported decreased fibrinolysis in euglobulin fractions from patients with APS, attributing this effect to impaired factor XII activation. β_2 GPI has also been reported to protect t-PA from inhibition by plasminogen activator inhibitor type 1 (PAI-1), an activity blocked by monoclonal aPL.⁸⁶

Conclusions

Annexin A2 plays an important role in the pathogenesis of the APS. It is an important binding site for β_2 GPI on the surface of endothelial cells and monocytes, and cross linking or clustering of annexin A2-bound β_2 GPI leads to cellular activation with expression of a procoagulant phenotype and inflammatory cytokines. This interaction may also depend upon

^aBu, *et al.*, unpublished observation 4/08.

TLR4, although whether β_2 GPI binds to TLR4 directly or only via annexin A2 or other cellular proteins is unknown. Similar mechanisms may operate on monocytes and endothelial cells, although the relative importance of MyD88-dependent versus MEK-1/ERK signalling pathways is uncertain. Additional work defining these pathways *in vitro* and *in vivo* is likely to yield new therapeutic insights for treatment of the APS. For example, it is possible that pharmacologically disrupting the interaction between β_2 GPI and annexin A2, β_2 GPI and TLR4, or the assembly of a signalling complex that contains these proteins, and perhaps others, may modulate the pathophysiologic signalling events responsible for acquisition of the procoagulant phenotype in patients with the APS. The roles of additional β_2 GPI binding proteins also must be considered when assessing the cellular responses to β_2 GPI/anti- β_2 GPI binding.⁸⁷

Annexin A2 and the annexin A2:S100A10 heterotetramer also play an important role in the regulation of cell surface fibrinolysis, and this process may be impaired by anti-annexin A2 antibodies.¹² The full impact of β_2 GPI on annexin A2-stimulated t-PA-mediated plasminogen activation has yet to be clearly defined. The fact that β_2 GPI alone may potentially stimulate this activity, and has been reported to enhance plasminogen activation following incubation with intact endothelial cells,⁸³ complicates this analysis. More detailed characterization of the nature of the annexin A2- β_2 GPI binding interaction may allow a more definitive investigation of this process.

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Figure 1.

Structure of the annexin A2:S100A10 heterotetramer. The S100A10 (p11) protein is shown binding to the annexin A2 tail domain. 1–4 represents the four annexin repeats within the core domain. Tyrosine and serine phosphorylation sites are present within the tail domain (from Brownstein, *et al.*¹⁷).

Figure 2. Activation of NFκB through engagement of TLR4 by LPS. This pathway involves MyD88, IRAK and Traf6, but not Traf2 (from Medzhitov and Janeway⁵⁴).

Figure 3.

Model of the annexin A2-S100A10 (p11) heterotetramer, depicting binding of tPA and plasminogen (PLG) leading to generation of plasmin (PLM). The anti-annexin A2 antibody (Antibody) might inhibit binding of tPA or PLG or block their interaction with each other. β_2 GPI might have a similar effect as the antibody, or perhaps directly stimulate plasminogen activation through annexin A2-dependent or independent interactions. P11 = S100A10; PLM = plasmin; tPA = tissue-type plasminogen activator; α_2 -PI = α_2 plasmin inhibitor. Illustration by A. Y. Chen (from McCrae⁸⁴).