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Review Article

Revisiting Beta 2 Glycoprotein I, the Major Autoantigen in the Antiphospholipid Syndrome

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ABSTRACT

Beta 2 glycoprotein I (β2GPI) is a single chain 50 kDa highly glycosylated glycoprotein at an approximate concentration of 4 μM in cells. The abundance of this protein in plasma and its high state of preservation indicate the important role of this protein in mammalian. In addition, β2GPI has a particular structure in the fifth domain, and is categorized as the major antigen recognized by autoantibodies present in antiphospholipid syndrome. Beta 2 glycoprotein I has been usually studied in the context of antiphospholipid antibody production. Complexes of β2GPI/anti-β2GPI antibodies have been examined in different coagulation and cell activation pathways. However, the function of β2GPI, independent from the antibodies, has not been clearly determined. In this paper different features of β2GPI including its structure, plasma concentration and its proposed in vitro and in vivo functions in clot formation and fibrinolysis along with anti-β2GPI antibodies (Abs) are discussed. Their inhibitory or promotive effects are delineated in each facet.

Keywords: Autoantibodies, β2 Glycoprotein I, Coagulation, Fibrinolysis

INTRODUCTION

Beta 2 glycoprotein I (β2GPI) was discovered in 1961 (1). However, increased interest in the function of this molecule ensued after determination of its amino acid sequence in 1984 (2).

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Beta 2 glycoprotein I was recognized as an adhesion molecule which may bind to phospholipids (3) such as phosphatidylserine (PS) on the surface of activated platelets and apoptotic bodies (4,5), and a number of negatively charged molecules such as heparin (6), DNA (7), lipoproteins (VLDL and LDL) (8,9), and intralipid (an artificial triglyceride emulsion) (10). In 1990s identification of β2GPI as a major target of antiphospholipid antibodies (11-13) opened a new direction to β2GPI studies. From that time β2GPI was investigated as a protein which might have a critical role in thrombosis, and both procoagulant and anticoagulant functions were referred to β2GPI based on in vitro assays. Increased interest in the function of β2GPI ensued after determination of its amino acid sequence and particularly its isolation as a necessary cofactor for the binding of patient antiphospholipid antibodies to phospholipids. A growing body of evidence has shown the effect of β2GPI on different aspects of clot formation, fibrinolysis, cell activation, immune response, atherosclerosis, apoptosis, angiogenesis and fetal loss (14,15). On the other hand, individuals deficient for β2GPI appear clinically healthy and do not show thrombotic or bleeding disorders (16,17). The physiological role of β2GPI may be, therefore, supportive or regulatory. However, Thrombin generation was significantly decreased in β2GPI +/- and -/- mice compared to the wild type indicating a possible procoagulant function of β2GPI in vivo (18). The intact β2GPI may be in balance with the cleaved or oxidized forms of the molecule in vivo (19-21). Alteration of this balance may contribute to perturbed regulatory activity of β2GPI and pathological consequences. On the other hand, studies on the in vivo thrombosis models have shown a prothrombotic effect for the infused anti β2GPI antibodies (22,23).

Beta 2 GPI Structure and Conformation in Plasma

Beta 2 glycoprotein I is a single chain glycoprotein consisting of 326 amino acid residues (2), containing 20% w/w heterogenous carbohydrates. It has a molecular weight of 54 KDa (24) and a plasma concentration of 150 to 300 μg/ml (25), and is one of the most abundant human plasma proteins being the second highest among the plasma proteins involved in clotting. Beta 2 glycoprotein I is highly expressed from the cells of the liver and placenta (26). It belongs to a super family of proteins characterized by repeating sets of ~60 amino acid residues, termed short consensus repeats (SCR) (27). Each SCR consists of 16 conserved residues and two fully conserved disulfide bonds that bridge the 1st to the 3rd and the 2nd to the 4th cysteine.

Domain V of β2GPI is Characterized by a Unique Composition of Amino Acids

In addition to the SCR region which is similar to domains I-IV, domain V contains an extra 7-residue insertion, a 19-residue C-terminal extension, and an additional disulfide bond which links the aforementioned regions and creates an unusual terminal loop for the molecule, rather than a usual free tail. The prominent charge in domain V is positive, and the included C281-KNKEKKC-C288 region, which is completely conserved is defined as

the phospholipid binding site of β2GPI (28). Domain V is cleaved by plasmin (29) and FXIa (activated FXI) (30) at Lys317-Thr318 which then fails to bind to phospholipids (31). The cleaved molecule is named clipped β2GPI.
The crystal structure of β2GPI shows a fishhook shape with no unpaired cysteines (27,32). However, we have recently published new data regarding the reduced form of β2GPI in plasma. Beta 2GPI may be reduced at Cys288-Cys326 (33) by thiol oxidoreductases secreted by platelets and endothelial cells (21,34). These findings show the participation of β2GPI in redox processes in vascular biology (15,35). In addition, new data is published regarding the existence of two different structures of β2GPI, circular and open forms, in circulation (36). The conformational change of the molecule may alter its role in coagulation and binding to patient antibodies.
Beta 2 glycoprotein I is recognized as the major target of autoantibodies detected in patients with antiphospholipid syndrome (APS) (11-13), a condition which exhibits as venous and arterial auto antibodies including anticardiolipin antibodies (aCL), anti-β2GPI antibodies and lupus anticoagulants (LA). The two formers are detected by ELISA, and the latter by clotting assays (14,37). Anti-β2GPI Abs are not only established as serological markers of APS (37), but also involved in the pathology of the syndrome (14). Clinical studies have shown the increased risk of thrombosis in patients with anti- β2GPI antibodies (38,39).

The Role of Beta 2 Glycoprotein I in Clot Formation

Beta 2 glycoprotein I has been shown to contribute to several pathways of the coagulation cascade, platelet activation and clot formation. However, the prominent effect of β2GPI as a procoagulant or anticoagulant factor, and the contribution of anti-β2GPI Abs in this effect has not been elucidated.

Beta 2 Glycoprotein I and Coagulation Pathway

Coagulation includes series of serine proteases that eventually lead to the generation of thrombin and the deposition of cross-linked fibrin polymers. Thrombus formation is believed to be initiated by the extrinsic pathway in vivo (40) while plasma FVII comes into contact with tissue factor (TF) which initiates blood clotting cascade by activating factors IX and X leading to thrombin generation. Beta 2 glycoprotein I is reported to have no direct effect on TF: FVIIa pathway in the presence of Phosphatidylserine/Phosphatidylcholine (PS/PhC) vesicles (41). However, it is believed that β2GPI in the presence of aCL/anti-β2GPI mAb may induce TF expression in endothelial cells and monocytes (42,43). It has been recently demonstrated that dimers of β2GPI may increase the size of fibrin clot in apolipoprotein E receptor 2 deficient mice and induce TF upregulation in peritoneal cells and homogenates of carotid arteries (44).
The *intrinsic pathway* of coagulation is triggered by contact activation of FXII followed by sequential proteolytic activation of prekallikrein, high molecular weight kininogen (HMWK) and FXI (40). Beta 2 glycoprotein I inhibits activation of prekallikrein (45) and

FXII (46) on chromogenic substrates in the presence of negatively charged phospholipids or on the surface of very low density lipoproteins (VLDL) (47). This inhibitory effect is independent, but may be amplified in the presence of anti-β2GPI IgGs. Beta 2 glycoprotein I binds FXI/FXIa in vitro and inhibits activation of FXI by thrombin and FXIIa (48) on the surface of activated gel filtered platelets.

Table 1. Beta 2 glycoprotein I and anti-β2GPI Abs in Coagulation.

Coagulation Factors	Author	In Vitro	B2GPI	α B2GPI Ab	Comments
Extrinsic pathway					
TF:FVII	Carson 88	+	[1]No effect	NI	[1]No effect on TF:FVII mediated activation of FX
TF	[1]Meroni 01 [1]Bohgaki 04 [2]Romany-Penabab 07	+	Induce	Induce	[2]β2GPI alone or [1]in complex with αβ2GPI mAbs induce TF expression
Contact pathway					
PK_a	Schousboe 88	+	[1]Inhibit	NI	[1]Inhibition of FXII mediated PK activation
$FXII_a$	Schousboe 95 McNally 96	+	[1]Inhibit	Inhibit	[1]Synchronized inhibition of FXII activation by β2GPI and α β2GPI IgG
FXI_a	[1]Shi 04 [2]Rahgozar 07	+	Inhibit	Inhibit	[1]β2GPI inhibits FIIa mediated activation of FXI , and [2]anti-β2GPI mAbs potentiate this effect
Common pathway					
FII_a	[1]Nimf 86 [2]Goldsmith 94	+	[1]Inhibit	[2]Inhibit	[1]β2GPI inhibits prothrombinase generation, and [2]anti-β2GPI IgGs potentiate this effect
FX_a	Shi 93 Galli 93	+	Inhibit	Inhibit	Lupus anticoagulants and some aCLs in complex with β2GPI inhibit tenase

αβ2GPI Ab=anti-β2GPI antibody, NI=not identified, $_a$=activation (ex: PK_a=Prekallikrein activation), TF=Tissue factor.
Numbers 1 and 2 link information between column 6, 4 and 5. Numbers written besides authors names show the date of paper publication (the last 2 digits of each date are mentioned).

Anti-β2GPI mAbs potentiate the inhibitory effect of β2GPI on thrombin mediated FXIa generation (49).
The *common pathway* is initiated by activation of FX through TF: FVIIa. Factor Xa, together with FVa forms the prothrombinase complex. The tenase complex may also be formed by factor IXa on the platelet surface. Platelet tenase generates more prothrombinase contributing to 'thrombin burst' and formation of a stable haemostatic plug (50). Beta 2 glycoprotein I inhibits prothrombinase formation on the surface of the activated platelets or phospholipid vesicles (4,51). This inhibition leads to less thrombin generation. It has been suggested that the inhibitory effect of β2GPI on prothrombinase is not due to direct interaction of occupied prothrombinase binding sites on the platelet surface (4). Anti-phospholipid IgGs in complex with β2GPI potentiate the inhibitory effect of β2GPI on prothrombinase generation (51). Moreover, Beta 2 glycoprotein I has been shown to inhibit generation of tenase on activated gel filtered platelets, and prevent in vitro FX activation (52). Lupus anticoagulants (52) and some aCLs (53) increase the inhibitory effect of β2GPI on FXa generation. Table 1, summarizes the effect of β2GPI on extrinsic, intrinsic and common pathways of coagulation in the presence or absence of anti-β2GPI Abs.

Beta 2 Glycoprotein I and Platelet Plug Formation

Platelet activation begins at the site of vascular injury with the exposure of collagen in the subendothelial matrix, leading to primary haemostasis, and continues by soluble agonists such as thrombin, ADP, and thromboxane A2 (54). Beta 2 glycoprotein I is reported to inhibit ADP-induced aggregation of gel filtered platelets (55). This effect was demonstrated specifically for ADP and did not affect thrombin-induced platelet aggregation. Moreover, the concentrations of β2GPI that were able to exert this inhibition were between 3.7 and 18 μM which is above β2GPI's physiological concentration.
Beta 2 glycoprotein I has been shown to inhibit 5-HT release during ADP-induced platelet activation (5). This inhibition is not dose dependent and affects specifically ADP, but not thrombin or collagen-mediated platelet release.
Beta 2 glycoprotein I has been previously introduced as the physiologic regulator of Von Willbrand Factor (VWF) in the body (56). A regulatory effect of β2GPI on platelet plug formation has been recently shown by two different groups based on the post-translational modifications of β2GPI. Hulstein and colleagues have demonstrated an inhibitory effect of β2GPI on platelet adhesion by competitively binding to the active VWF (56). Anti-β2GPI IgGs compete with this interaction contributing to increased levels of circulating active VWF in APS patients (56).These experiments were performed using the purified form of the molecule, which was oxidized during purification. On the other hand, our group has recently demonstrated that, the reduced form of β2GPI binds to VWF and increases platelets adhesion to VWF in a thiol exchange reaction. This function is mediated by the glycoprotein Ib alpha complex and the thiol oxidoreductases on the platelet surface generate the reduced form of β2GPI (33,34). A summary of β2GPI and anti-β2GPI Abs effects on platelet response is depicted in Table 2.

Table 2. Beta 2 glycoprotein I and anti-β2GPI Abs in Platelet activation, adhesion and aggregation.

Platelet Response	Author	In Vitro	B2GPI	α B2GPI Ab	Comments
Adhesion	Hulstein 07	+	[1]Inhibit	Potentiate	[1]β2GPI, in contrast to anti- β2GPI IgGs, inhibits interaction of active VWF with platelets
	Passam 10	+	Potentiate		
Release	Nimpf 87	+	Inhibit	NI	β2GPI inhibits ADP-mediated 5-HT release. This effect is not dose dependent
Aggregation	Nimpf 85	+	[1]Inhibit	NI	[1]β2GPI *inhibits ADP-mediated platelet aggregation [2]β2GPI promotes thrombin mediated plt aggregation and serotonin release
	Rahgozar08	+	[2]Potentiate		

NI=not identified, *=the concentration of β2GPI used in this experiment was above its physiological concentration
Numbers 1 and 2 link information between column 6, 4 and 5. Numbers written besides authors names show the date of paper publication (the last 2 digits of each date are mentioned).

Beta 2 Glycoprotein I and Regulation of Clot Formation

Coagulation is regulated by protein C pathway, tissue factor pathway inhibitor (TFPI), and a number of serine Protease Inhibitors (serpins) such as antithrombin III (ATIII), heparin cofactor II (HCII) and protein Z-dependent protease inhibitor (ZPI) (50) which serve to restrict excessive clot formation or thrombosis.

Table 3 summarizes the effects of β2GPI on regulation of clot formation in the presence or absence of anti-β2GPI Abs. Except for protein S activity, β2GPI may inhibit the regulating factors of coagulation. This may suggest a procoagulant effect for β2GPI on fibrin clot formation. The regulatory role of β2GPI on serpins is appealing, and exhibits a new property of β2GPI in coagulation and fibrinolysis. The effect of β2GPI on second messengers of the platelet response needs further delineation (57-72).

Considering the above data, a negative feedback loop by which β2GPI and its clipped form (c β2GPI) may control the coagulation pathway is proposed (Figure 1). This pathway is initiated by the procoagulant activity of β2GPI with regard to protecting thrombin from inactivation by HCII/Heparin (73). Beta2GPI bound thrombin, which is therefore resistant to HCII/Heparin, may generate FXIa contributing to thrombus propagation.

Table 3. β2GPI I/anti-β2GPI Abs and regulation of clot formation.

Regulatory Factors	Author	In vitro	B2GPI	α B2GPI Ab	Comments
Coagulation					
PC_a	[1]Keeling 93 [2]Atsumi 98 [3]Smirnov 95 [3]Cariou 88 [3]Freyssinet 86	+	[1]Inhibit	Inhibit	[2]αPL bind to PC in the presence of β2GPI and [3]inhibit PCa
APCactivity	[1]Mori 96 [2]Matsuda 95 Izumi 02 Safa 05	+	[1]Inhibit	[2]Inhibit	[1]β2GPI blocks APC mediated inhibition of FVa. [2]APC-R is β2GPI dependent and requires Ab divalency
PrS	Walker 93 Merrill 99	+	[1]Potentiate	Inhibit	[1]β2GPI inhibits PrS-C4BP interaction and potentiates the cofactor activity of PrS for APC
ZPI	Forastiero 03	+	Inhibit	[1]Inhibit	[1]anti phospholipid IgGs potentiate the inhibitory effect of β2GPI on ZPI activity
TFPI	Salemink 00 Lean 06	+	Inhibit	[1]Inhibit	[1]anti-β2GPI IgGs inhibit TFPI inhibition of FXa generation in the presence of β2GPI
PCI	Cucnik 04	+	[1]NI	NI	Coexistence with PCI while isolation from plasma
HCII	Rahgozar 08	+	[1]Inhibit	Inhibit	[1]β2GPI inhibits inactivation of FIIa by HCII in the presence of heparin
Platelet Response					
Adenylate cyclase	Schousboe 80	+	Inhibit	NI	β2GPI inhibits adenylate cyclase activity
cGMP	Nimpf 85	+	Potentiate	NI	β2GPI increases cGMP acting as an anti-platelet agent

a=activation (ex: PC_a=Protein C activation), αCL=anti-cardiolipin antibody, APC = activated protein C, APC-R=APC resistence, PrS=protein S, C4BP=C4b-binding protein, ZPI=protein Z-dependent inhibitor, TFPI=tissue factor pathway inhibitor, HCII=heparin cofactor II.
Numbers 1 and 2 link information between column 6, 4 and 5. Numbers written besides authors names show the date of paper publication (the last 2 digits of each date are mentioned).

On the other hand, FXIa cleaves β2GPI at Lys317-Thr318, abrogating its protective effect on thrombin, therefore suppressing its procoagulant activity. Beta 2 glycoprotein I is depicted as a Janus-headed protein in Figure 1 which may illustrate both procoagulant and anticoagulant effects. Interestingly, the anticoagulant effect of β2GPI concerning inhibition of FXI activation may contribute to suppressing cleavage of β2GPI by FXIa, hence shifting to a procoagulant activity in this system. In pathological circumstances, anti-β2GPI Abs may interfere with the β2GPI's regulatory pathway and amplify the procoagulant activity of the protein by potentiating the protective effect of β2GPI on thrombin leading to prothrombotic tendencies.

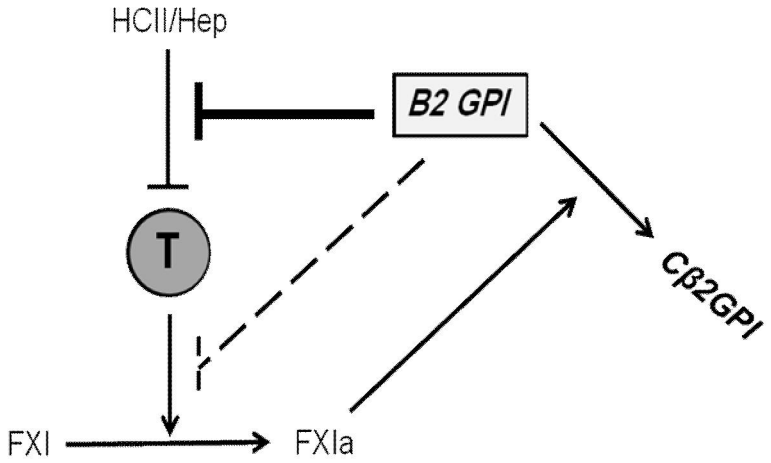

Figure 1. The regulatory role of β2GPI/cn-β2GPI in the coagulation pathway.
Beta 2 glycoprotein I protects thrombin from the inhibitory effect of HCII/Heparin. This procoagulant effect of β2GPI is shown using thick dark lines. Thrombin activates FXI, FXIa cleaves β2GPI, and cleavage abrogates the procoagulant activity of β2GPI. The anticoagulant role of β2GPI is also shown using dashed lines. Cβ2GPI=clipped native β2GPI, HCII=heparin cofactor II, Hep=heparin, T = thrombin, ⊣ = inhibitory effect, → = stimulatory effect.

Beta 2 Glycoprotein I and Fibrinolysis

A growing body of evidence shows impaired fibrinolysis in patients with APS (74-77). Beta 2 glycoprotein I, independent from the aPLs, is demonstrated to not only influence the clot formation, but also affect clot removal. Fibrinolysis is downregulated by serine protease inhibitors and anti-plasmins which may be affected by β2GPI.
Fibrinolysis is the physiological disintegration of fibrin to restrict and resolve blood clots (78). Fibrin serves as a cofactor for plasminogen to which it binds and converts it to plasmin. In the *extrinsic pathway of fibrinolysis*, this happens in the presence of the serine

protease tissue plasminogen activator (tPA), and endothelial cell secretory product. Yasuda et al showed that clipped (cn-) β2GPI but not the intact molecule suppresses the extrinsic pathway of fibrinolysis by inhibiting tPA mediated plasmin generation (79). The strong correlation between elevated plasma levels of cn-β2GPI and plasmin/plasmin inhibitor complex (PPI) in patients with ischemic stroke (79) supports the in vivo regulatory effect of β2GPI on extrinsic fibrinolysis. In contrast to this study, Lopez-lira et al showed direct interaction of the intact β2GPI with plasminogen which may be competitively inhibited by epsilon amino caproic acid (EACA). It should be considered that in these studies the method of purification has not been identified and the purity of β2GPI has not been assessed in reduced conditions. The proteolytic cleavage of β2GPI may happen easily during purification of β2GPI using heparin-Sepharose affinity chromatography (20), thereby affecting the results of the study.
Plasminogen may also become activated by pro-urokinase expressed by epithelial cells, monocytes, and some tumor cells (the *intrinsic pathway of fibrinolysis)* (80,81) or non eukaryotic plasminogen activator such as *streptokinase (SK)* (82). SK is fibrin independent (83), and generates plasmin explosively in the bloodstream at sites distant from the fibrin clot (82). It is suggested that β2GPI inhibits the intrinsic fibrinolytic activity (84). A human and a murine anti-β2GPI mAb potentiated the inhibitory effect of β2GPI on the intrinsic fibrinolysis (84). In contrast Beta 2 glycoprotein I is demonstrated to potentiate SK activated fibrinolysis (85).

Beta 2 Glycoprotein I and Regulation of Fibrinolysis

Fibrinolysis is controlled by plasminogen activator inhibitor (PAI), antiplasmins such as α2-antiplasmin and α2-macroglobulin, and thrombin activatable fibrinolysis inhibitor (TAFI). Regulation of fibrinolysis is predominantly performed through inhibition of tPA.
Ieko et al. demonstrated that β2GPI suppresses PAI mediated inactivation of tPA (86). This effect was phospholipid independent, measured by using a system in which β2GPI was incubated with tPA in the presence of plasminogen and soluble fibrin. Plasmin generation was assessed by chromogenic assay and considered as an indicator of tPA activity.
Two human monoclonal anti-β2GPI antibodies (mAbs), EY2C9 and EY1C8, inhibited the protecting effect of β2GPI on tPA inactivation of PAI, suggesting the antifibrinolytic and prothrombotic contribution of aPls in patients with APS (86). The inhibitory effect of mAbs on β2GPI impact on PAI is shown to be β2GPI and phospholipid dependent (86).

CONCLUSION

Beta 2 glycoprotein I is an abundant glycoprotein of the body, and its unique amino acid composition has provided specific properties for this protein in different fields including coagulation and fibrinolysis. The mechanisms by which β2GPI exerts its function is not clearly identified. *Cleavage* of the protein at Lys317-Thr318 by plasmin or FXIa may be accounted as one of the mechanisms by which β2GPI regulates fibrinolysis or possibly

coagulation. The other mechanism might be *dimerization or multimerization* mediated by anti-β2GPI Abs. A growing body of evidence shows that anti-β2GPI Abs does not block β2GPI function, but in many cases, promote the physiological effect of β2GPI. It has been demonstrated that *oxidation* of β2GPI may alter interactions of the molecule with phospholipid surfaces, modulate recognition by anti-β2GPIAbs (87), and mediate cell activation (88). Oxidation might be another mechanism by which β2GPI exerts its role in diverse pathways. Structural biochemical studies of β2GPI deserve close inspection and may open new insights into the physiology of β2GPI.

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