SCIENTIFIC REVIEW

β2-GLYCOPROTEIN I AND ANTI-β2-GLYCOPROTEIN I ANTIBODIES: WHERE ARE WE NOW?

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SUMMARY

β2-Glycoprotein I (β2-GPI), a plasma protein with in vitro anticoagulant properties, has been recognized to have an important role in the antiphospholipid syndrome (APS) as a cofactor and an (co)antigen in ELISA assays. Although β2-GPI levels were found to be increased in some patients with APS, the clinical value of measuring β2-GPI levels in APS is not known. Several reports have suggested that anti-β2-GPI antibodies may be a marker for the APS and might be more specific for the vascular complications of the APS than anticardiolipin antibodies. There have been major discoveries about phospholipid (PL) and antibody binding sites on β2-GPI, although more studies are needed. Reports of changes in cell membrane PL composition or exposure of other anionic molecules by apoptosis, cell activation and oxidative injury suggest mechanisms to explain β2-GPI binding and the generation of cryptic epitopes for aPL/anti-β2-GPI antibodies.

KEY WORDS: β2-Glycoprotein I, Anti-β2-glycoprotein I, Systemic lupus erythematosus, Antiphospholipid syndrome, Primary antiphospholipid syndrome.

It is now accepted that a form of primary antiphospholipid syndrome (APS) exists that is distinguishable from systemic lupus erythematosus (SLE) [1]. Although this distinction has proved useful on clinical and therapeutic grounds, there is considerable evidence to support a hypothetical common pathway(s) in the generation of relevant antibodies in both clinical conditions (Table I). However, in the past 6 yr it has been appreciated that antiphospholipid (aPL) antibodies may require crucial co-factors, amongst which β2-glycoprotein I (β2-GPI) is the best studied to date.

β2-GPI was first described 35 yr ago [8]. However, the importance of this plasma protein only became recognized widely following the identification of its role in the APS as a cofactor for the binding of certain antibodies to anionic phospholipids (PL) as detected in solid-phase ELISA [9–13]. In the main, these antibodies are found in patients with autoimmune disease, but as a recent study has shown (following parvovirus infection), they can occur in individuals with infectious disease [14]. Substantial efforts have been made to identify the complex mechanism(s) and relevant antigen(s) of APS. A variety of antibodies linked to different clinical syndromes are now recognized (Tables II and III) [15, 16]. Recent information about other antigens and co-factors like phosphatidylethanolamine-kininogen is leading to further changes in classification [17, 18].

Attempts to classify the immunoglobulins participating in ‘antibody-associated thrombosis’ might need further revision because of nosological difficulties [e.g. pseudo-aPL antibodies, hidden anticardiolipin (aCL) antibodies, true aPL antibodies] and the recent recognition of additional antibodies (e.g. antibodies to fibrin-bound tissue-type plasminogen activator [19] and heparin [20]). Other important risk factors for thrombosis, like Factor V Arg506-Gln mutation [21] and plasma homocysteine levels [22], should also be taken into account when evaluating affected patients. To discuss all the possible patho-

TABLE I

SLE and APS: Analogy of clinical features and antibody production in these two conditions

| 1. | The strongest linked disease association of APS is with SLE |
| 2. | The prevalence of aPL antibodies and APS in patients with SLE increases with disease duration [2] |
| 3. | Some patients classified initially as having primary APS subsequently developed SLE [3] |
| 4. | The clinical expression and some laboratory abnormalities of the APS in patients with primary APS, and SLE plus APS, are similar [4] |
| 5. | The main serological analogy between SLE and APS is the fact that characteristic clinical features are associated with high-titre IgG immunoglobulins (anti-dsDNA in lupus nephritis and aCL in APS thrombosis) |
| 6. | Anti-DNA antibodies (albeit mostly anti-ssDNA) were found in more than half of the patients with primary APS [5] |
| 7. | The comparison of the sequences of the IgG and IgM aCL antibodies has disclosed that the former tend to carry more somatic mutations than their IgM counterparts, which is similar to IgG anti-DNA antibodies. The importance of antigen-driven mechanisms in the generation of both antibodies is thus evident [6] |
| 8. | The predominant subclass of anti-β2-GPI is IgG2 both in patients with SLE and primary APS [7] |

SLE, systemic lupus erythematosus; APS, antiphospholipid syndrome; aCL, anticardiolipin; β2-GPI, β2-glycoprotein I.

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Other antiphospholipid antibodies?

1. Lupus anticoagulant antibodies (recognize PT–PL complex)
2. Anticardiolipin antibodies (recognize β2-GPI–PL complex)
   - Type A (have anticoagulant activity)
   - Type B (devoid of anticoagulant activity)
3. Anti-free-β2-GPI antibodies
4. Anti-protein S and activated protein C (APC) antibodies (recognize protein S and APC after their binding to anionic PL)
5. True antiphospholipid antibodies (do not need plasma ‘cofactors’)
6. Other antiphospholipid antibodies?

PT, prothrombin; PL, phospholipid; β2-GPI, β2-glycoprotein I.

Classification of antiphospholipid antibodies according to Alarcon-Segovia and Cabral [16]

**True aPL**
1. IgM antiphosphatidylcholine (aPC) in all strains of mice
2. IgM aPC in human autoimmune haemolytic anaemia
3. Hidden IgG antiphospholipid antibodies (aCL) in normal humans
4. aCL in syphilis
5. Monoclonal aCL from humans

**Pseudo-aPL**
1. aCL in human autoimmune diseases
2. Some lupus anticoagulants (LAC)
3. Anti-phosphatidylethanolamine
4. Anticofactor antibodies
   - Anti-β2-glycoprotein I
   - Anti-prothrombin (LAC)

Molecular structure and function of β2-GPI

β2-GPI is a protein of ~50 kDa found at a concentration of ~200 μg/ml in plasma, 40% of which is found associated with lipoproteins of various classes [8, 23]. This level is independent of age or sex [24]. It is a highly glycosylated single-chain polypeptide of 326 amino acids containing a high proportion of proline and cysteine residues [25].

β2-GPI has five common repeating motifs/domains of ~60 amino acids with a highly conserved pattern of cysteine residues. It is thus classified as a member of the Short Consensus Repeat or Complement Control Protein (CCP) superfamily [26]. The first four domains are typical examples of this superfamily, while the fifth (containing 82 amino acid residues) is aberrant, containing two additional cysteines and a long C-terminal tail.

In vitro, β2-GPI inhibits thrombin formation at two different levels: contact activation and prothrombinase activity [27, 28]. Excessive formation of thrombin and an increased risk of thrombosis are associated with a deficiency in β2-GPI. The prevalence of β2-GPI deficiency in the normal population is relatively high (~10%). Bancsi et al. [24] tried to determine whether a hereditary β2-GPI deficiency is a risk factor for (familial) thrombophilia. They identified β2-GPI deficiency in 10 out of 129 healthy individuals and in four groups of patients with thrombophilia, and found that the prevalence of the deficiency was very similar in all groups (6.8–12.5%). They concluded that β2-GPI deficiency in itself is an unlikely risk factor for the development of thrombosis. This may be because other haemostatic regulatory factors can compensate in β2-GPI-deficient individuals. However, the physiological role of this molecule in haemostasis, in vivo, is as yet undetermined. A summary of some of the in vitro properties of β2-GPI is given in Table IV.

The discovery that some ‘aCL’ antibodies recognize a complex of cardiolipin (CL) and β2-GPI, or β2-GPI alone, in vitro has led to the study of serum β2-GPI levels. There is considerable interest in the possible role of this plasma protein in the pathogenic mechanism of thrombosis associated with aPL antibodies.

Several questions about the role of β2-GPI in the development of thrombosis remain unanswered. Are plasma β2-GPI levels decreased in patients with aCL antibodies because the plasma protein is being consumed by the formation of immune complexes? Is there a relationship between levels of serum aPL/aCL antibodies and β2-GPI? Do levels of plasma β2-GPI reflect symptoms related to SLE and APS? In attempting to answer these questions, one needs to keep in mind the possibility that the functional activity of β2-GPI may be compromised by aPL/aCL antibodies without any demonstrable change in its plasma levels.

<table>
<thead>
<tr>
<th>Author [reference]</th>
<th>Effect of β2-glycoprotein I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schousboe [27]</td>
<td>Inhibition of the contact activation of the intrinsic coagulation pathway</td>
</tr>
<tr>
<td>Nimpf et al. [28]</td>
<td>Inhibition of platelet prothrombinase activity</td>
</tr>
<tr>
<td>Nimpf et al. [29]</td>
<td>Inhibition of ADP-induced platelet aggregation</td>
</tr>
<tr>
<td>Henry et al. [30]</td>
<td>Inhibition of activation of factor XII by kaolin or ellagic acid</td>
</tr>
<tr>
<td>Schousboe [31]</td>
<td>Inhibition of factor XIa-mediated activation of prekallikrein</td>
</tr>
<tr>
<td>Shi et al. [32]</td>
<td>Inhibition of factor Xa generation by platelets</td>
</tr>
<tr>
<td>Schousboe and Rasmussen [33]</td>
<td>Inhibition of autoactivation of factor XII in plasma*</td>
</tr>
<tr>
<td>Mori et al. [34]</td>
<td>Inhibition of the factor Va-dependent prothrombinase complex and potentiation of thrombin generation in the presence of activated protein C</td>
</tr>
<tr>
<td>McNally et al. [35]</td>
<td>Inhibition of factor XII activation on very-low-density lipoprotein</td>
</tr>
</tbody>
</table>

*Reaction is also inhibited by anti-β2-glycoprotein I antibodies.
$\beta_2$-GPI can bind other negatively charged macromolecules other than PL, including heparin, platelets and DNA [36, 37], although DNA binding could not be confirmed in a recent study [38]. In addition to its effects on coagulation, $\beta_2$-GPI might be part of a buffer mechanism to interfere with the development of an autoimmune response against neoepitopes, including epitopes generated from foreign (bacterial) antigens like membranes or DNA [39]. However, this mechanism itself might be a target for an aberrant immune system. A recent in vitro study suggested a lack of anticoagulant effect of $\beta_2$-GPI under near physiological conditions with a relatively weak binding to plasma membranes [40]. This finding is evidence that $\beta_2$-GPI is part of a system that operates principally in abnormal conditions of oxidative stress and inflammation. The possible antiatherosclerotic effect of $\beta_2$-GPI via macrophage–ox-LDL interaction may be another example of its role [41].

**$\beta_2$-GPI: CLINICAL CONNECTIONS**

Cohnen [42] was the first to note a slightly increased concentration of serum $\beta_2$-GPI in 10 SLE patients, compared to healthy adults.

Since then, numerous reports of $\beta_2$-GPI antigen levels in patients with aPL antibodies have produced conflicting results, with normal, elevated and decreased levels reported (as reviewed in Table V).

$\beta_2$-GPI levels were, however, relatively higher in SLE patients with hyperlipidaemia compared with healthy controls [43]. This finding demonstrated that the increased $\beta_2$-GPI levels shown in the original study by Cohnen [42] may have been due to a secondary hyperlipidaemic state, perhaps caused by lupus nephritis and/or prednisolone treatment which can increase $\beta_2$-GPI levels in SLE patients [43]. Previously, Takamatsu et al. [51] had reported high $\beta_2$-GPI levels in patients with hyperlipidaemic states. Hyperlipidaemia is associated with a high risk of thrombosis. So is there a double risk of thrombosis where both increased $\beta_2$-GPI levels and hyperlipidaemia occur? Apparently not, as Ichikawa’s results [43] show that $\beta_2$-GPI levels are not correlated with thrombotic events in patients with SLE or APS. However, long-term follow-up may be needed to resolve this issue.

An association between primary hyperlipidaemia and elevated $\beta_2$-GPI levels has also been established by McNally et al. [52]. Patients with primary hyperlipidaemia have been shown to have significantly higher mean $\beta_2$-GPI levels compared to controls, suggesting a direct relationship between plasma lipoprotein and $\beta_2$-GPI levels. $\beta_2$-GPI distribution between the lipoprotein fractions is altered in hyperlipidaemic patients.

When comparing APS and SLE (aCL-negative, non-APS) patients, mean $\beta_2$-GPI levels were significantly higher in the patients with APS [44, 45]. The sera with high $\beta_2$-GPI levels tended to express high IgM anti-phosphatidylethanolamine (aPE) and IgG aCL reactivities. In accordance with Ichikawa’s study [43], Galli et al. [46] also reported no relationship between $\beta_2$-GPI levels and a thrombotic history. However their results, for reasons which are unclear, conflict in that they have found that patients with aCL antibody-positive/lupus anticoagulant (LA)-negative serology have normal plasma levels of $\beta_2$-GPI. Patients with a positive LA (aCL antibody positive or negative) were compared with patients without LA (aCL antibody positive or negative) by

**TABLE V**

Reports of $\beta_2$-glycoprotein I plasma levels

<table>
<thead>
<tr>
<th>Author</th>
<th>$\beta_2$-GPI levels</th>
<th>Study groups</th>
<th>aPL/aCL (+) or (−)</th>
<th>Association between $\beta_2$-GPI and thrombotic history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohenen [42]</td>
<td>†*</td>
<td>SLE (n = 10)</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Ichikawa et al. [43]</td>
<td>†* (P &lt; 0.05)</td>
<td>SLE (n = 36)</td>
<td>(+) (n = 18)?</td>
<td>No</td>
</tr>
<tr>
<td>Vlachoyiannopoulos et al. [44, 45]</td>
<td>† (P &lt; 0.05)?†‡</td>
<td>APS (n = 12)</td>
<td>(+)</td>
<td>?</td>
</tr>
<tr>
<td>Galli et al. [46]</td>
<td>Normal*</td>
<td>LA (−) (n = 9)</td>
<td>(+)</td>
<td>No</td>
</tr>
<tr>
<td>De Benedetti et al. [47]</td>
<td>†* (P = 0.001)</td>
<td>LA (+) (n = 34)</td>
<td>(+) or (−)</td>
<td>?</td>
</tr>
<tr>
<td>McNally et al. [48, 49]</td>
<td>Normal*†‡</td>
<td>LA (+) (n = 26)?</td>
<td>(+) or (−)</td>
<td>?</td>
</tr>
<tr>
<td>Rai et al. [50]</td>
<td>Normal†</td>
<td>SLE, primary APS</td>
<td>(+) (n = 17)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SLE, primary APS</td>
<td>(+) (n = 57)</td>
<td>?</td>
</tr>
</tbody>
</table>

$\beta_2$-GPI, $\beta_2$-glycoprotein I; SLE, systemic lupus erythematosus; APS, antiphospholipid syndrome; LA, lupus anticoagulant; aPL, antiphospholipid antibody; aCL, anticardiolipin antibody.

†, no data available.
‡, No difference between aPL/aCL (+) or (−) groups.
$\beta_2$-GPI levels compared to normal controls.
$\gamma$, compared to normal controls.
De Benedetti’s group [47]. They showed no difference in β2-GPI levels between the patient groups and controls, and no relationship with aCL antibody or LA levels. Thus, there are conflicting data with respect to a possible relationship between β2-GPI levels and LA activity.

Thrombosis in patients with aCL antibodies might be consequent upon the inhibition of the antithrombotic properties of β2-GPI by these autoantibodies. Furthermore, decreased β2-GPI levels would be anticipated in patients with disseminated intravascular coagulation (DIC) due to consumption of the available β2-GPI supply. Matsuda et al. [53] collected samples at the time of initial diagnosis and throughout the course of DIC, and monitored β2-GPI levels. One patient’s β2-GPI level was normal, but the mean of the seven patients with DIC studied was significantly lower than in the normal controls. During DIC episodes, β2-GPI levels fell further in six patients and remained low in five of the seven patients studied, despite treatment for DIC. Accelerated consumption of β2-GPI during the course of DIC was deemed the most likely cause of the decrease in β2-GPI levels seen in these patients, together with other coagulation factors.

Approximately 30–40% of plasma β2-GPI is found to be associated with lipoproteins while ~10% is in an apparently free form [23, 48]. There was no significant difference between free β2-GPI levels in normal controls and patients with lupus, irrespective of their aPL antibody status. However, total β2-GPI levels of the aPL antibody-positive SLE group were increased significantly compared to the aPL antibody-negative SLE and normal control groups [48]. This observed increase was a result of increased amounts of bound β2-GPI (β2-GPI complexed with other plasma constituents such as lipoproteins, and in immune complexes). Increased total β2-GPI levels were associated with a clinical history of thrombosis and/or fetal loss in the patients studied. This suggests that β2-GPI may play a role in the pathogenic mechanism of thrombosis associated with aPL antibodies [49]. All the patients in this study had a normal plasma lipid profile so that elevated levels of β2-GPI due to hyperlipidaemic states could be excluded.

In women with recurrent miscarriage, β2-GPI concentrations were found to be normal. Rai et al. [50] compared 57 aPL antibody-positive women and 57 age-matched aPL antibody-negative women with normal controls who had children, but no previous history of miscarriage or thrombosis. β2-GPI concentrations were similar in the aPL antibody-positive and -negative women with recurrent miscarriage and the normal controls. Most interestingly, the aPL antibody-positive women had normal β2-GPI levels, which contradicts previous reports of increased β2-GPI levels in conjunction with the presence of aPL [46, 49]. The functional activity of β2-GPI may be compromised by aPL antibodies without any demonstrable change in plasma levels.

The studies reviewed show no consensus as to whether plasma levels of β2-GPI reflect symptoms related to APS, or whether levels are influenced by the presence or absence of aPL antibodies.

ANTI-β2-GPI ANTIBODIES: DETECTION AND CLINICAL ASSOCIATIONS

Galli et al. [10] first reported that affinity-purified aCL antibodies can bind directly to the cofactor-coated plates and speculated that aCL antibodies are directed to cofactor rather than PL. Initially, this binding could not be uniformly confirmed and it was hypothesized that the target antigen could be the PL-β2-GPI complex, a cryptic/neoepitope generated by the interaction of these two components [9].

Arvieux et al. [54], using a β2-GPI ELISA, reported a good correlation between aCL and anti-β2-GPI antibodies. Purified aCL antibodies were also shown to bind β2-GPI-coated plates in the absence of PL, but not β2-GPI in the fluid phase [55]. Viard et al. [56] reported that anti-β2-GPI antibodies were associated with thrombosis and LA in patients with SLE. However, contradictory results appear in the literature, possibly because of methodological differences [57]. Although Koike et al. [58] first showed that aCL antibodies bound to β2-GPI when irradiated plates were coated with this antigen, not all irradiated plates appear to have this effect [57]. Finally, it was shown that aCL antibodies bound directly to β2-GPI coated on irradiated plates (with electrons or γ-ray), and X-ray photoelectron spectroscopy revealed the oxidation of the surfaces of these plates [59]. Some investigators reported the successful detection of antibodies using non-irradiated [60], chemically treated [61] or polyvinylchloride plates [62]. These findings supported the view that a conformational epitope is generated when PL and β2-GPI interact, and some anti-β2-GPI antibodies recognize this neoepitope. The addition of β2-GPI antigen to ELISA systems aimed at inhibiting anti-β2-GPI antibody binding produced controversial results showing no inhibitory effect [59] or significant inhibition with a dose–response effect [56, 63], which may indicate a heterogeneous antibody population. Few human monoclonal aCL antibodies (all IgM) that bind to β2-GPI have been described to date [38]. Their binding to β2-GPI was not inhibited by fluid-phase β2-GPI.

Roubey et al. [64] proposed that anti-β2-GPI antibodies require a relatively high-density immobilized antigen which can be achieved by using irradiated plates. They found that purified IgG aCL antibodies from patients with APS bound to β2-GPI coated onto γ-irradiated polystyrene were dependent upon high antigen density and antibody bivalency. This property was ascribed to the low intrinsic affinity of these antibodies to β2-GPI. The weak binding of anti-β2-GPI antibodies to β2-GPI in the fluid phase supported this view. Although these findings did not exclude the conformational epitope concept, the observation that monovalent antibody fragments do
not bind significantly to β2-GPI on irradiated plates supports the importance of antigen density [64].

In a recent study comparing different methods of detecting anti-β2-GPI, 100% correlation between Western blot, dot-blot and ELISA methods was reported [65]. The results with recombinant and purified human β2-GPI used as an antigen in these assays were also identical. Purified IgG anti-β2-GPI antibodies from patients with APS were studied and shown to be low-affinity, mainly monoreactive autoantibodies that can bind soluble β2-GPI [66]. Some authors favour the use of carbonate buffer as a coating buffer, which allows higher amounts of antigen binding than phosphate-buffered saline, and filtration of the test sera to decrease non-specific background [63].

McNally et al. [67] were unable to find anti-β2-GPI antibodies in patients with active infection, including patients with transiently positive aCL antibody serology. In some studies, patients with syphilis and positive aCL antibody serology were reported as negative for anti-β2-GPI antibodies [60, 68], although a larger study on secondary syphilis sera reported that 29% of patients had these antibodies [63]. In patients with SLE, IgG anti-β2-GPI antibodies were shown to be associated more strongly with manifestations of APS than aCL antibodies [49, 65, 69], but in some series the prevalence was <50% [70]. Balestrieri et al. [63] found an association of IgG anti-β2-GPI antibodies with recurrent fetal loss, and of IgM anti-β2-GPI antibodies with a history of thrombosis and thrombocytopenia. Recently, anti-β2-GPI antibodies were found to be associated with recurrent fetal loss without an identifiable cause [71].

Many patients with primary APS also have IgG anti-β2-GPI antibodies [49, 60, 62, 69], but in some series the prevalence was <50% [70]. Interestingly, in these cross-sectional studies, there were some patients with SLE and primary APS who lacked IgG aCL antibodies but were positive for IgG anti-β2-GPI antibodies, and vice versa [49, 60, 63, 65]. When adsorption with CL micelles was undertaken on sera from patients with the primary APS who had aCL and anti-β2-GPI antibodies, the aCL antibody activity was inhibited, but the inhibition was significantly less prominent for anti-β2-GPI antibody activity, although the latter could still be detected by the dot-blot method [54]. Another group suggested that six patients with manifestations of APS who were repeatedly negative for aPL antibodies, but had IgG anti-β2-GPI antibodies, constituted a serologically distinct primary syndrome [72]. They referred to some patients with SLE who had the same serological profile. However, this discrepancy between aCL and anti-β2-GPI antibody assays was not confirmed by Amengual et al. [69], who raised the question of the possible effect of different methods of human β2-GPI antigen preparation.

The discrepancy between anti-β2-GPI and aCL antibody assays may be explained partially by species-specific anti-β2-GPI antibodies, because in an aCL antibody assay bovine β2-GPI is present (in the blocking and dilution buffers) and the amount of β2-GPI in the system is uncontrolled, which differs from commercial aCL antibody kits [73]. Arvieux et al. [74] compared human and bovine β2-GPI as an antigen in patients positive for anti-β2-GPI antibodies, and reported that IgM binding to β2-GPI was significantly decreased when bovine β2-GPI was used and that this binding showed no change with time. There was no difference in the IgG isotype and Cabral et al. [72] demonstrated that their patients’ sera were also positive when they used bovine β2-GPI as the antigen. Thus, the species specificity of anti-β2-GPI cannot explain all of the discrepancies. The finding that purified IgG anti-β2-GPI from patients with APS can recognize β2-GPI from different species supports this view [66].

Two recent studies have addressed the value of anti-β2-GPI antibody as a marker for the clinical features of APS. In both studies, the positive predictive value of anti-β2-GPI antibodies for a history of thrombosis was found to be higher than that of aCL antibodies [75, 76]. Anti-β2-GPI antibodies were found to be highly specific, but not sufficiently sensitive for a history of thrombosis. An analysis of sequential samples from seven patients with SLE from our own cohort, who had had thrombotic/neurological events, showed that in six cases IgG anti-β2-GPI antibodies were evident several months before these events occurred (M. Inanc, S. Donohoe, C. T. Ravirajan, E. L. Radway-Bright, I. Mackie, S. Machin and D. A. Isenberg, unpublished observations). Prospective longitudinal data are needed for a more precise risk assessment.

**EPITOPE MAPPING OF β2-GLYCOPROTEIN I**

Early work by Steinkasserer et al. [77] identified a highly positively charged sequence in the fifth domain which is constrained by a disulphide bridge, Lys282–Lys287. This is likely to be a surface-exposed turn, using the secondary structure model for the CCP domain proposed by Barlow et al. [78], and they have postulated that this sequence may constitute the binding site for negatively charged PL on the β2-GPI molecule.

Hunt et al. [79] identified a region in the fifth carboxy terminal of β2-GPI critical for its lipid binding and aCL antibody cofactor activity. This group has shown that β2-GPI cleaved and disulphide linked at Lys317–Thr318 completely loses its ability to bind negatively charged phospholipids and to act as an aCL antibody cofactor.

Hunt and Kriji [80] have gone further by showing that the sequence Cys281–Lys–Asn–Lys–Glu–Lys–Lys–Cys288 in the fifth domain is the major PL binding site of β2-GPI. Inhibition experiments using synthetic peptides have revealed that the PL binding site is located in peptides containing the sequence...
Cys281–Cys288. The results show that the highly positively charged sequence Lys282–Lys287 requires the flanking Cys281 and Cys288 residues present for PL binding. They postulate that these Cys residues are critical for the appropriate conformation of the PL binding sequence and, therefore, for binding of β2-GPI to negatively charged phospholipids in vitro. This highlights both the importance of this Cys281–Cys288 sequence and the tertiary structure of the molecule in PL binding.

More evidence that Cys281–Cys288 is the major PL binding site of β2-GPI has been provided, using synthetic peptides containing this sequence [81]. The peptides inhibit the binding of 125I-labelled β2-GPI to CL, and thus blocked autoimmune aCL antibodies reacting in a modified aCL antibody ELISA, but how does the Lys317–Thr318 ‘clip’ affect β2-GPI binding to anionic PL and its cofactor activity? The clipped residues have been shown to be in close proximity to the Cys281–Cys288 sequence [82]. Kandiah et al. [81] postulated that the region on either side of the ‘clip’ becomes more mobile, thus interacting with residues in the phospholipid binding region, and interfering with phospholipid binding.

Autoimmune aCL/anti-β2-GPI antibodies do not bind to the ‘clipped’ form of β2-GPI (Cys317–Thr318 clipped). The ‘clip’ apparently causes a steric change in the protein which disrupts the binding of the antibodies to it. Some peptide sequences from the fifth domain of β2-GPI have been shown to have cofactor activity, while others are bound in free solution by human monoclonal aCL antibodies [83].

In 1996, Igarashi et al. [84] produced five β2-GPI mutant genes in which various domain regions have been deleted, and the resulting incomplete β2-GPI molecules were expressed in a baculovirus system. Their evidence suggests that the PL binding site exists only in the fifth domain. The mutant proteins have also been used to determine the domains on β2-GPI containing epitope(s) recognized by aCL antibodies. Unexpectedly, their data suggest that the epitope(s) is not on the fifth domain, and propose that the binding of PL to the fifth domain is required for exposure of the cryptic epitope on another domain, possibly the fourth. Thus, the precise epitope(s) recognized by aPL antibodies on the β2-GPI molecule remain to be determined.

β2-GPI AS AN AUTOANTIGEN AND GENERATION OF ANTI-β2-GPI

Animal experiments

Experiments by Rauch and Janoff [85] provided the first evidence of the immunogenic potential of β2-GPI. They reported that β2-GPI, when injected with CL, not only acted as a co-immunogen required for the production of aCL antibodies in Balb/c mice, but after injection of β2-GPI with or without CL, anti-β2-GPI antibodies were produced. In contrast, mice immunized with CL alone did not produce aCL antibodies.

Immunization of NIH/Swiss mice and NZW rabbits with β2-GPI in Freund’s complete adjuvant also resulted in anti-β2-GPI and aPL antibody production without cross-reaction [86]. These results were confirmed by another group using lipid-free adjuvants to avoid lipid contamination of the β2-GPI [87]. Following the immunization of a rabbit with β2-GPI, distinct sets of antibodies binding to β2-GPI alone and negatively charged PL and β2-GPI were identified [88]. In the same study, affinity-purified antibodies from patients with APS reacted similarly to the rabbit antibodies. Ionic interactions may be important in explaining the binding of some autoimmune aPL antibodies to anionic PL in the absence of β2-GPI [89]. Kouts et al. [88] suggested that charge-dependent binding to β2-GPI/PL might be an epiphenomenon associated with some charged aPL antibodies, and anti-β2-GPI antibodies may cross-react with PL as a result of electrostatic interactions. This cross-reaction could be detected as aPL activity in the ELISA. Recently, it was shown that immunization of the mice with a PL-binding viral peptide, that has a structural homology with the PL-binding region of β2-GPI, induced β2-GPI-dependent aPL antibodies [90].

PATHOGENIC ROLE(S) OF ANTI-β2-GPI ANTIBODIES: INTERACTION WITH COAGULATION, ENDOTHELIUM AND PLATELETS

The question as to whether antibodies produced in mice after immunization with β2-GPI are truly pathogenic remains unresolved. Although there were methodological differences, Blank et al. [91] were able to show that these mice had a high percentage of fetal resorptions and thrombocytopenia, but Silver et al. [92] reported that in spite of the antibody production, these clinical findings did not differ significantly from control mice. They commented that at least some of these antibodies were not pathogenic and/or the antibodies generated by this immunization protocol differed from pathogenic aCL antibodies from patients with APS [93, 94].

More recently, Garcia et al. [95] reported a high rate of fetal resorption in PL/J mice that had been immunized with β2-GPI. Intriguingly, three animals (out of four) kept for long-term follow-up developed transverse myelitis and histology revealed a large thrombus compressing the spinal cord and some thrombi in smaller vessels.

Coagulation

The interaction of factor XII, prekallikrein and high-molecular-weight kininogen is needed for contact activation of the coagulation cascade on a suitable surface. It was demonstrated that β2-GPI has an inhibitory effect on factor XII [30] and factor XIIa mediated activation of prekallikrein [31]. Removal of β2-GPI from plasma with LA activity was associated with the loss of the anticoagulant activity [96]. The correlation between anti-β2-GPI
antibodies and LA was demonstrated in clinical studies [56,60,97]. Rabbit polyclonal and mouse monoclonal antibodies to β2-GPI were found to inhibit thrombin generation in a dose-dependent manner, and this inhibition was due to interaction with β2-GPI [98]. Murine monoclonal antibodies to β2-GPI were shown to have LA properties by Arvieux et al. [99]. Taken together, these findings indicate that β2-GPI is an important factor in LA activity.

Anti-β2-GPI antibodies may also have a role in activated protein C resistance [100]. Recently, PL-mediated autoactivation of factor XII was shown to be inhibited by β2-GPI and anti-β2-GPI antibodies in a synchronized manner [33]. Factor XII activation on triglyceride-rich lipoproteins was also inhibited by β2-GPI, and a regulatory role for β2-GPI in haemostatic reactions occurring on plasma lipids was suggested [101]. This inhibitory property was affected by affinity-purified IgG from a patient with a high level of anti-β2-GPI activity. The information about the direct role of anti-β2-GPI on coagulation in patients with APS is limited, especially by the heterogeneity of antibody populations, and the relationship between these antibodies and thrombosis remains to be elucidated.

Endothelium

Endothelial cells (EC) and platelets are important sites of action for antibodies that have a role in the pathogenesis of thrombosis. aPL and anti-endothelial cell antibodies (aECA) are probably distinct antibody populations that frequently co-exist [102], and it has been shown recently that anti-β2-GPI antibodies are also associated with aECA in patients with APS [103]. Previous studies suggested that there is a cross-reaction between some populations of aPL antibodies with EC, which can be enhanced by perturbation of cell membranes with paraformaldehyde fixation [104]. β2-GPI was shown to bind EC monolayers in vitro [102] and, in vivo, EC of vessels inside the placental villi in patients with APS [105].

Le Tonguèze et al. [106] reported binding of purified aCL antibodies to Eahy926, a human endothelioma cell line, in a β2-GPI-dependent manner. Del Papa et al. [107] also showed that the binding activity of anti-β2-GPI antibody-positive sera to EC was dependent on β2-GPI. They further proved the binding of affinity-purified anti-β2-GPI antibodies and human IgM monoclonal antibodies recognizing β2-GPI to EC, via β2-GPI [102]. More interestingly, they reported that these affinity-purified anti-β2-GPI antibodies activate EC, which was demonstrated by induced E-selectin expression [107].

Activation of EC by aPL antibodies in a β2-GPI-dependent manner was also confirmed by showing increased monocyte adhesion to EC and increased secretion of E-selectin, vascular cell adhesion molecule-1 and intracellular adhesion molecule-1 [108]. On the other hand, EC activated by cytokines were not found to increase the binding of β2-GPI [104]. Activation of EC induced by aPL antibodies has not been confirmed by all groups, and the binding of β2-GPI and aCL antibodies to resting or cytokine-activated EC has not been demonstrated [109].

Taken together, current data indicate that EC membranes may be an important site of action for autoimmune aCL antibodies, anti-β2-GPI antibodies and β2-GPI. Exposure of a procoagulant surface for binding of β2-GPI, containing negatively charged PL or other anionic structures (e.g. heparan sulphate, thrombomodulin), seems to be the prerequisite for the reaction with relevant antibodies [110]. This exposure may be the result of EC activation or apoptosis, both can be caused by aPL antibodies or antibodies other than aPL/anti-β2-GPI antibodies. The finding that aECA can trigger the expression of anionic PL on EC [111], and anti-DNA/anti-RNP antibodies can cause apoptosis in lymphocytes [112], may support the hypothesis of a concerted action of different antibodies in the pathogenesis of APS.

Platelets

Several antigenic targets for aPL antibodies were described on platelets, including platelet-activating factor, platelet glycoprotein IIIa and β2-GPI [110]. Exposure of anionic PL after activation leads to the binding of β2-GPI to platelet membranes, which possibly mediates aPL/anti-β2-GPI antibody binding [113]. Although it was suggested that antibodies that bind to β2-GPI can oppose the inhibitory effect of this molecule on factor Xa generation, activated platelet surfaces and platelet-derived microparticles [114], the pathogenic role of aPL antibody–platelet–endothelial cell interaction in vivo, in the pathogenesis of APS, is still uncertain.

CONCLUSION

The identification of β2-GPI as a cofactor in the binding of aCL antibodies, and its subsequent more accurate description as a (co)antigen, has had a substantial influence on our understanding of the APS. Analogous to the various clinical syndromes of ‘antibody-associated thrombosis’, autoantibodies involved in the pathogenesis are obviously diverse. Although several assays have been described to investigate APS, the available methods that can be used for clinical purposes are limited. Encouraging data on anti-β2-GPI antibody measurement justifies wider use of this test as a marker of the APS together with LA and aCL antibody assays. There is, however, an obvious need for standardization. Questions about the physiological role of β2-GPI remain unanswered and research in this area is limited mostly to information from in vitro studies. Considerable advances have been achieved in the understanding of antigen–antibody interaction in APS, but full details of the structural and functional characteristics of anti-β2-GPI antibodies remain to be determined.
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