Immunologic characterization and functional properties of murine antibodies raised against deleted mutants of human β₂-glycoprotein I

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Keywords: autoimmunity, antibodies, β₂-glycoprotein I, antiphospholipid syndrome, platelets

Abstract

β₂-Glycoprotein I (β₂GPI) is a 50 kDa molecule proposed as a principal target of ‘autoimmune’ antiphospholipid antibodies (aPL). We have used deleted mutants (DM) representing different domains of β₂GPI (I–IV, IV–V and V) for immunization of naive mice and studied the characteristics of the respective murine IgG preparations in comparison with affinity-purified IgG from two patients with primary antiphospholipid syndrome. Immunization with β₂GPI and with the DM produced anti-β₂GPI antibodies, part of which reacted with negatively charged phospholipids (PL), whereas reactivity with cardiolipin was evident only in the IgG from mice immunized with β₂GPI. These results are consistent with the presumption that aPL are induced following the in vivo association of β₂GPI (used for immunization) with resident negatively charged PL. Accordingly, DM which either lack the PL binding site or aPL attachment locus did not elicit, upon immunization, antibodies reactive with PL. Further, murine anti-β₂GPI IgG and human ‘autoimmune’ aPL were similar, albeit not identical, in terms of DM requirement for PL binding and charge dependency. Murine antibodies and human aPL, regardless of their binding characteristics, were found to bind significantly to platelets upon their activation with thrombin and to promote platelet activation. The results of the current study emphasize the dissimilarities between human ‘autoimmune’ aPL and murine anti-β₂GPI. Thus, anti-β₂GPI antibodies to different DM as well as human aPL are capable of binding and activating human platelets provided β₂GPI is present.

Introduction

β₂-Glycoprotein I (β₂GPI) is a 50 kDa protein cofactor required for the binding of ‘autoimmune’ antiphospholipid antibodies (aPL) to negatively charged phospholipids (PL) (1–3). This protein is a member of the complement control protein family containing five (I–V) short consensus repeats (‘sushi domains’). In recent years, intensive work has been carried out to elucidate the nature of the interaction between PL, β₂GPI and aPL, with the aim of gaining insight into the pathogenesis of the prothrombotic state in the antiphospholipid syndrome (APS). It has been suggested that ‘autoimmune’ aPL are directed against a cryptic epitope exposed on the structure of β₂GPI, following its association with negatively charged synthetic surfaces (4) or PL (5). Alternatively, β₂GPI may support the binding of aPL from ‘autoimmune’ sera due to its increased density, facilitated by the interaction with PL (6).

Autoimmune aPL are heterogeneous in terms of binding properties and while some react directly with β₂GPI, others cross-react with negatively charged PL (7,8). There is no consensus on the clinical significance of specific subsets of

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Transmitting editor: I. Pecht
Received 24 December 1996, accepted 4 March 1997
aPL and anti-β2GPI antibodies with regard to their association with the manifestations of APS, probably reflecting a multifactorial etiopathogenesis of this yet incompletely understood syndrome.

Recently, it has become apparent that the fifth domain of β2GPI is responsible for the attachment to PL (9,10); however, controversy still exists regarding the location of epitope(s) which provide the cofactor activity for the binding of ‘autoimmune’ aPL to β2GPI or alternatively to the complex formed following its association with PL (1,11).

In an attempt to resolve some of these enigmas, human β2GPI was used for immunization of animals resulting in the production of antibodies which have been claimed to mimic, in their binding properties, human ‘autoimmune’ aPL (12–15).

The recent construction of deleted mutants (DM) of β2GPI expressed by a baculovirus system (16–17) has prompted us to explore the immune reaction following immunization of mice with these proteins. Herein we provide a description of the binding characteristics of antibodies to three of these DM in comparison with affinity-purified antibodies from two patients with the primary APS. Furthermore, the binding properties of these antibodies are complemented by a study of their binding and activating properties in a human platelet system in the absence and with addition of β2GPI.

Methods
Antigens
Recombinant DM representing different domains of β2GPI (DM I–IV, IV–V and V) were used for mouse immunization (17). Human β2GPI was purified from the serum of a healthy adult as previously described (12).

Mice
Female BALB/c mice (6–8 weeks old) were purchased from the Sackler Faculty of Medicine, Tel-Aviv University, Israel.

Patients
Two patients with primary APS were selected for the comparative studies. Both patients were diagnosed by the occurrence of thromboembolic events, recurrent fetal losses and high titers of IgG aPL, part of which were reactive with β2GPI.

Immunization protocol
Mice were actively immunized in their hind footpads with 10 µg of the following DM: whole molecule (WM), I–IV, V, IV–V and V, emulsified in complete Freund’s adjuvant. Three weeks later, a booster immunization was given at the same area, with 10 µg of the respective preparation dissolved in PBS. The mice were bled from their retro-orbital plexuses at monthly intervals.

Purification of mouse IgG
IgG from pooled mouse sera from all study groups was purified by Protein G-Sepharose column (Pharmacia, Uppsala, Sweden). Absence of contamination of the IgG preparation with β2GPI was confirmed by immunoblot with mouse anti-β2GPI antibodies using enhanced chemiluminescence; demonstrated in Fig. 1.

Affinity purification of human aPL
Affinity purification of human aPL was performed as previously described (18) with slight modifications. First, 5 ml of human sera was mixed with 2.5 ml of phosphatidylserine micelles in 0.15 M NaCl (3 mg/ml) and the mixture was incubated at 37°C for 1 h and 4°C overnight with constant mixing. The mixture was then centrifuged at 30,000 g for 15 min and the supernatant was removed. The washing procedure was repeated twice. After the third wash, the pellet was resuspended in 1 ml of 1 M NaI and incubated for 1 h with gentle mixing. Then, 0.5 ml of chloroform was added, and the mixture mixed for 1 min and allowed to stand. The mixture was separated into two layers and the top aqueous layer containing anti-phosphatidylserine antibodies was recovered and dialyzed against PBS overnight. The dialyzed extract containing antibodies was purified further using Protein G-Sepharose chromatography. Absence of contamination with β2GPI of human antibodies was confirmed using the method described in Fig. 1.

Anti-phospholipid ELISA
A modified ELISA to determine reactivity of the mouse and human antibodies to PL was performed as previously described (17). Briefly, microtiter plates (Nunc, Maxisorp, Denmark) were coated either with an anionic PL [cardiolipin (CL), phosphatidylserine (PS) and phosphatidylinositol (PI)] and phosphatidylcholine (PC), all from Sigma (St Louis, MO), at a concentration of 50 µg/ml dissolved in ethanol, except for PC (chloroform:methanol, 1:3). Plates were dried under vacuum and blocked with TBS containing 0.5% gelatin. The plates were then washed three times with TBS and different concentrations of mouse or human IgG preparations were added to the wells treated with human β2GPI (5 µg/ml for 30 min) or 0.1% gelatin/TBS alone. The binding of the antibodies was detected with goat anti-mouse or anti-human IgG alkaline phosphatase conjugate and the addition of substrate (p-nitrophenylphosphate). The results were expressed as absorbance at 405 nm (OD405).

Fig. 1. Absence of contamination with β2GPI of human and mouse IgG preparations. The human and mouse IgG preparations were evaluated by SDS–PAGE and Western blotting with affinity-purified rabbit anti-β2GPI polyclonal antibody (absorbed on human or mouse IgG–Sepharose). For detection of the bands the horseradish peroxidase-conjugated anti-rabbit IgG combined with enhanced chemiluminescence were used. Lanes 1–2, 50 ng of affinity-purified human anti-β2GPI antibodies; lanes 3–4, 50 ng of affinity-purified mouse anti-β2GPI antibodies. Lanes 5–9, purified human β2GPI (at concentrations of: 100, 20, 4, 2 and 1 ng respectively).
Aliquots of 1 µg/ml β2GPI or different DM were incubated overnight in 50 mM bicarbonate buffer, pH 9.6, at 4°C in 96-well polystyrene plates (Nunc). After three washings with TBS, blocking was performed with 0.5% gelatin/TBS for 2 h (as for the anti-PL ELISA). The plates were then washed three times and human or murine IgG (diluted in 0.1% gelatin/TBS) was then added and incubated for 2 h at room temperature. After three washings, alkaline phosphatase conjugated goat anti-human IgG or anti-mouse IgG respectively diluted in 0.1% gelatin/TBS (1:10,000) was added for 2 h. After an additional three washings, the substrate p-nitrophenylphosphate in a sodium carbonate buffer (pH 9.8) was added and absorbance was read at 405 nm.

Effect of DM in the modified anti-CL ELISA
CL (50 µg/ml in ethanol) was coated on plain polystyrene 96-well plates (Nunc) and allowed to evaporate at 4°C overnight. After three washings with TBS, the wells were blocked with 0.5% gelatin/TBS for 2 h at room temperature. The plates were washed and different DM (WM, I–IV, V or I–III) were added at different concentrations (2.5–15 µg/ml) for 30 min at room temperature. Following the addition of the IgG, bound antibodies were detected using 1:10,000 dilution of goat anti-human or anti-mouse IgG conjugated to alkaline phosphatase (Sigma) and the addition of its substrate p-nitrophenylphosphate. Color was read in an ELISA reader (SLT Laboratory Instruments, Vienna, Austria) at 405 nm.

Immunoblot assays and detection of murine IgG reactivity with different DM
Different DM (WM, I–IV, I–III, II–V, II–IV, III–V and V; 2 µg/ lane) were subjected to SDS–PAGE using 12% acrylamide separating gel. The separated proteins were transferred onto nitrocellulose sheets by a constant current of 2.5 mA/cm² for 1 h. The sheets were then incubated with TBS, pH 7.4, containing 1% bovine serum albumin (Sigma). After three washings with TBS/0.05% Tween 20 and 1% BSA. Individual sheets were incubated with mouse IgG (aWM, al–IV and alV). Following further washings, the sheets were incubated with peroxidase-conjugated goat anti-mouse IgG (Jackson Immuno Research, USA) and developed with 4 chloro-1-napthol and H₂O₂.

Preparation of platelet suspension for FACS
Venous blood was drawn from healthy donors, who had not used any medication for the previous 10 days, into 15% ACD (0.73% citric acid, 2.2% sodium citrate, 2.45% dextrose, pH 4.5). Platelet-rich plasma (PRP) was prepared by centrifuging the blood at 180 g for 15 min. Prostaglandin E₁ (PGE₁, 100 ng/ml) was added to the PRP to inhibit platelet release and aggregation during subsequent procedures. PRP was then centrifuged at 1200 g for 10 min. The resulting platelet pellet was resuspended in Tyrode's buffer (137 mM NaCl, 2.8 mM KCl, 0.4 mM NaH₂PO₄, 2H₂O, 12 mM NaHCO₃, 5 mM glucose, 0.35% BSA and 5 mM EDTA, pH 6.5). Platelets were washed twice in the same buffer in the presence of PGE₁. Finally they were resuspended in Tyrode's buffer without PGE₁ and EDTA (pH 7.4) to which 1 mM calcium chloride was added.

FACS analysis for assessment of binding and activation of human platelets with mouse and human IgG
Washed platelets were diluted with Tyrode's buffer (pH 7.4) to 2.5×10⁷/ml. Then, 40 µl of the platelet suspension was incubated for 10 min with or without 5 µl of β2GPI (80 µg/ml), 5 µl of α-thrombin (0.05 U/ml) and 5 µl of anti-P-selectin IgG conjugated to phycoerythrin. Then, 5 µl of mouse IgG (80 µg/ml) β2GPI or 5 µl of IgG from two APS patients was added and the incubation was continued for 30 min. Platelets were washed twice and resuspended in 90 µl of Tyrode's buffer. Then, 10 µl of goat anti-mouse IgG or goat anti-human IgG conjugated to FITC was added and incubation was repeated for 30 min. Subsequently, platelets were washed and resuspended in 0.6 µl of 1% formaldehyde in Tyrode's buffer for FACS analysis. The stained antibodies were used at saturating conditions and isotype-matched antibodies were used as a non-specific control. From each sample, 5000 platelets were analyzed using an EPICS-XL Coulter flow cytometer. The results were expressed as mean fluorescence intensity obtained in three or four independent experiments.

Results
Binding of mouse and human IgG to β2GPI and PL
The purified IgG preparations obtained from mice immunized with the different recombinant preparations of β2GPI and from patients with primary APS reacted both with PL and with β2GPI coated on the plates. As shown in Fig. 2(A), the IgG of mice immunized with WM reacted with anionic PL (mean OD ± SD, n = 5; 1.450 ± 0.150) and significantly less with neutral PL (mean OD ± SD, n = 5; 0.323 ± 0.022) (Fig. 2B). IgG to DM I–IV and V displayed negligible binding to negatively charged PL and to neutral PL (OD ± SD, n = 5; 0.054 ± 0.002 and 0.064 ± 0.003 respectively), whereas reactivity with β2GPI was clearly evident (mean OD ± SD, n = 5; 1.116 ± 0.106). Affinity-purified aPL from the patients with primary APS reacted with both negatively charged PL (mean OD ± SD, n = 5; 0.599 ± 0.010) and β2GPI (mean OD ± SD, n = 5, 0.565 ± 0.022) (data not shown).

Effect of different DM on the binding of mouse aWM and human aPL to PL
The addition of native β2GPI enhanced the binding of mouse aWM IgG (Fig. 3A) and human aPL (Fig. 3B) to CL. Addition
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of both DM I–IV and I–III had an inhibitory effect on the binding of mouse aWM IgG to CL-coated plates, whereas no effect on the binding of human aPL to PL was noted. DM V had no effect on the binding of mouse aWM to CL, yet inhibited the binding of human aPL in the respective assay.

Binding of mouse antibodies to DM by ELISA and immunoblot

By employing ELISA, it was observed that binding of aWM (Fig. 4A) was significant to all DM (which represent all the domains within β₂GPI). The al–IV (Fig. 4B) and aV (Fig. 4C) bound the domains which correspond to the preparation used for immunization. By immunoblotting, the binding of aWM (Fig. 5A) is clear to nearly all DM (and WM). A similar binding pattern was evident regarding the al–IV (Fig. 5B) with clear reactivity to all DM containing domains I–IV (with no binding to the V domain). aV (Fig. 5C) displays clear binding to all DM containing the V domain.

Competition for binding of human affinity-purified aPL to β₂GPI and CL-coated plates by mice IgG

No competition of mouse IgG with affinity-purified human aPL was observed by use of the modified anti-CL ELISA or the β₂GPI ELISA. The assay was employed also in 0.15 M NaCl buffer to eliminate the binding of charge-dependent mouse and human antibodies with no significant competition (data not shown).

Effect of ionic strength on antibody binding

The charge dependencies of mouse IgG preparations and human aPL from the patient with primary APS were determined by using buffers with different ionic strength in the solid-phase assays (Fig. 6A and B). The binding capacities of mouse and human antibodies were tested in modified anti-CL ELISA and anti-β₂GPI ELISA. The binding of mouse aWM, as well as the binding of al–IV and aV to β₂GPI-coated plates was not affected by the addition of 1.5 M NaCl buffer (mean OD ± SD, n = 5; 1.116 ± 0.023 versus 1.205 ± 0.010, 1.362 ± 0.053 versus 1.328 ± 0.022 and 0.669 ± 0.100 versus 0.564 ± 0.033 respectively), whereas the binding of affinity-purified IgG from the patients was decreased by 20% (mean OD ± SD, n = 5; 0.935 ± 0.045 versus 0.743 ± 0.005). Binding in the anti-CL ELISA of aWM, al–IV and aV was diminished in the presence of 1.5 M NaCl (mean OD ± SD, n = 5; 0.645 ± 0.018 versus 0.227 ±
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0.050, 0.411 ± 0.028 versus 0.131 ± 0.013, 0.449 ± 0.017 versus 0.144±0.009 respectively), whereas binding of human aPL to CL was inhibited by 18.5% (mean OD ± SD, n = 5; 0.594 ± 0.025 versus 0.484 ± 0.030).

Effect of thrombin and β2GPI on binding of murine anti-β2GPI IgG to human platelets and P-selectin expression

The binding of mouse antibodies and control mouse IgG to resting and thrombin-preactivated platelets was assessed by FACS. In parallel, P-selectin expression on the platelet membrane was assayed. The results were obtained from three independent experiments, one of which is presented in Fig. 7. Slightly increased binding to platelets of mouse IgG to the different DM was evident as compared with IgG from a normal mouse (Fig. 7A). The binding of mouse IgG was followed by a moderate increase of P-selectin expression, suggesting activation of the platelets. Following platelet activation with thrombin

Fig. 4. Binding of mouse and human IgG to DM-coated plates by ELISA. ELISA plates were coated with the different mutants (1 µg/well) and the mouse and IgG preparations were added as described in Methods (A, aWM; B, aI–IV; C, aV).

Fig. 5. Immunoblot assays for detection of murine IgG reactivity with different DM. Different DM (1, WM; 2, I–IV; 3, I–III; 4, II–V; 5, II–IV; 6, III–V; 7, V; 2 µg/well) were subjected to SDS-PAGE as described in Methods. Individual sheets were incubated with IgG from mice immunized with DM (A, aWM; B, aI–IV; C, aV).
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Fig. 6. The effect of ionic strength on the binding of mouse IgG and affinity-purified human aPL in the anti-β2GPI and modified anti-CL ELISA. Different salt concentrations (0.15 and 1.5 M NaCl in PBS) were used to assess the effect of ionic strength on the binding of murine IgG and affinity-purified IgG from a representative patient with APS. The binding of the IgG was assayed employing the anti-β2GPI (A) and modified anti-CL ELISA (B).

Fig. 7. Effect of thrombin and β2GPI on binding of murine anti-β2GPI IgG to human platelets and P-selectin expression. Washed platelets were preincubated with normal mouse IgG (1) or mouse IgG to different DM (2, I–IV; 3, IV–V; 4, V) or to whole molecule (5) of β2GPI in the presence of: Tyrode’s buffer (A), α-thrombin (B), β2GPI (C) or with both α-thrombin and β2GPI (D). At the preincubation time, anti-P-selectin–phycoerythrin IgG was added to all tubes. After washing, anti-mouse IgG–FITC was added. Platelets were incubated again, washed, fixed with 1% formaldehyde and subjected to FACS.

Fig. 8. Binding of human aPL IgG to normal platelets (A) and P-selectin expression (B). Washed normal platelets were preincubated with α-thrombin, β2GPI and anti-P-selectin-phycoerythrin IgG as described in Methods. Then IgG from healthy humans (N) or patients with APS (1 and 2) were added. After incubation and washing, anti-human IgG–FITC was added. Platelets were washed, fixed with 1% formaldehyde and analyzed by FACS.

Binding of human aPL IgG to platelets and P-selectin expression

As shown in Fig. 8, in the presence of thrombin and β2GPI, increased binding to platelets of human aPL derived from two patients was evident by FACS and expressed as mean fluorescence intensity (4.89 ± 0.62 in the patient 1; 3.43 ± 0.28 in patient 2 as compared with 1.33 ± 0.27 in the normal volunteer, P < 0.001). At the same time, P-selectin expression on normal platelets was significantly increased only by the IgG of the first patient (2.29 ± 0.38 in patient 1; 1.69 ± 0.19 in patient 2 as compared with 1.36 ± 0.17 in the control sample).
Discussion

The purpose of the present study was to characterize the polyclonal immune response to different preparations of \( \beta_2 \)GPI in solid-phase and \textit{in vitro} human platelet assays with special reference to human ‘autoimmune’ aPL. We presumed that the study of the polyclonal response to the different DM, despite its heterogeneous nature (as compared with mAb), could represent more convincingly the diverse spectrum of autoantibodies in the sera of patients with APS. We have confirmed the presence of two populations of antibodies in response to immunization with the complete structure of \( \beta_2 \)GPI, i.e. ‘pure’ anti-\( \beta_2 \)GPI antibodies and antibodies cross-reactive with negatively charged PL (Fig. 2A). However, IgG obtained following immunization with DM I–IV and V was not cross-reactive with CL, implying that the complete structure of \( \beta_2 \)GPI was not cross-reacted with CL-ELISA, whereas binding to \( \beta_2 \)GPI-coated plates was of higher avidity. Human aPL displayed moderate charge dependency both in the modified anti-CL and anti-\( \beta_2 \)GPI ELISA, suggesting that some of the \( \beta_2 \)GPI reactivity may not be specific (Fig. 6).

Several authors have suggested that the immune response induced following immunization of rabbits (14) and mice (12–13,15) with human \( \beta_2 \)GPI resembles the autoantibody pattern found in humans with APS. We have thus chosen representative patients with primary APS and applied similar assays to study the characteristics of their given antibodies and compared them with the immune response towards the DM. Indeed, the pattern of antibodies found in the patients resembled mostly that found in the mice immunized with human \( \beta_2 \)GPI. However, competition assays between murine anti-\( \beta_2 \)GPI antibodies and affinity-purified human aPL did not reveal similar binding specificities, probably reflecting either the heterogeneous nature of antibodies found in the sera of patients with APS or the differences in the binding characteristics of mouse and human antibodies. An additional difference between human ‘autoimmune’ aPL and mouse aWM was found with regard to their binding to the different DM. Hence, \( \beta_2 \)GPI unexpectedly enhanced the binding of both murine (aWM) and human (aPL) antibodies to PL. DM I–IV and I–III did not display similar enhancement in humans, probably due to the lack of a PL binding site residing in the fifth domain. The inhibition of binding of mouse anti-\( \beta_2 \)GPI antibodies to PL could be explained by the capture of the latter IgG by the unbound DM I–IV (lacking the V domain) as was also evident by the ELISA and immunoblot assays (Figs 4 and 5). This binding prevented the detection of mouse antibodies which remained complexed in the fluid phase. Supporting this presumption is the similar inhibition obtained using DM I–III.

The solid-phase studies complemented by the inability to obtain competition between mouse and human antibodies imply that antibodies induced following immunization of mice with human \( \beta_2 \)GPI are not identical to human ‘autoimmune’ aPL, despite several similar characteristics.

The role of aPL in the pathogenesis of APS has received considerable attention in recent years, as manifested by the establishment of several animal models (15,19–21). In a very recent study (22), the authors have shown that antibodies elicited following immunization of mice with \( \beta_2 \)GPI possess thrombogenic properties, evident in a ‘pinch-injury’ murine model. These results reinforce previous data suggesting that immunization of naive mice with human \( \beta_2 \)GPI results in a clinical picture resembling human APS, manifested by thrombocytopenia, increased fetal loss and prolonged activated thromboplastin time (15).

Special emphasis has been put on platelets as possible targets of ‘pathogenic’ aPL, considering both the occurrence of thrombocytopenia and a prothrombotic state in APS (23–26). Platelet assays bear several advantages relevant to the current study. (i) \textit{In vitro} assays could be performed in the absence and the presence of \( \beta_2 \)GPI, thus dissecting its role in the binding and activation of platelets by aPL. (ii) Platelets are prototypic cells which (upon activation) display loss of membrane asymmetry, with partial ‘scrambling’ of lipids, the result of which is the exposure on the outer surface of the negatively charged PL concomitant with the internalization of the positively charged phosphatidylserine (27). The exposure of PS upon platelet activation has been suggested to provide a negatively charged surface, thus promoting aPL binding in a \( \beta_2 \)GPI-dependent manner (28). This system seemed appropriate for the current study by providing an established in vitro assay that could be correlated with the solid-phase studies and furnish a fair additional tool for the comparison of human and murine aPL.

Despite the previously described dissimilarities in the solid-phase assays, platelet studies have shown that human and all murine IgG preparations possessed binding and activating properties. In the washed platelet system (where \( \beta_2 \)GPI is depleted from the medium), the binding and platelet activation potential of mouse antibodies was nearly negligible and could either be attributed to minor activation of the manipulated platelets or to the basal binding of the antibodies to neutral PL (Fig. 2B). However, with the addition of \( \beta_2 \)GPI, enhancement of platelet binding and activation (by mouse antibodies and human aPL) occurred manifested by P-selectin expression upon their surface. The \( \beta_2 \)GPI-dependent increased binding and activation of platelets was marked when mouse IgG (to DM and to WM) was assayed and not with control IgG, thus supporting a pronounced interaction of the antibodies with human platelets. It is of interest that aV and aI–IV IgG, which could be taken to represent two ‘opposing’, non-overlapping populations (as can be observed by the lack of spreading of autoimmune response evident in the ELISA and immunoblot assays) were both capable of binding and activating human platelets with similar intensity (Fig. 7). Platelet activation induced by thrombin resulted in increased binding of all mouse IgG (including control) to platelets (Fig. 7B). The integrative explanation of the functional assays is that the mouse antibodies specific for the native structure of \( \beta_2 \)GPI or to DM (displaying high affinity) bound \( \beta_2 \)GPI complexed with
PS exposed on the surface of the preactivated platelets. This binding could also be observed in the solid-phase assays (modified anti-CL ELISA). Human aPL represent a more heterogeneous population; their binding to β2GPI is less pronounced and despite these different binding properties they were able to bind and activate human platelets (Fig. 8), similar to the murine antibodies.

The results of this study show that immunization with different domains of β2GPI results in restricted antibody production by the mouse which is specific for the given antigen. Charge-dependent cross-reactive anti-CL antibodies were induced only when the mice were immunized with native β2GPI but not with DM lacking domains I–IV or V. aW2M could be distinguished from human ‘autoimmune’ aPL by their solid-phase binding properties. Despite the differences in the immunological profile of mouse IgG, they were equally potent in promoting platelet binding and activation as were human aPL. It will be of particular interest to investigate if the different pathways of platelet activation are merely the result of binding to β2GPI and whether these truly represent multiple mechanisms of inducing the prothrombotic predisposition in APS.

Acknowledgements

The work was supported by the Israel–Japan binational grant no. 6113, the Ministry of Sciences, Israel.

Abbreviations

\[\beta_2\text{GPI} \quad \beta_2\text{-glycoprotein I}
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\[\text{al–IV} \quad \text{antibodies to DM I–IV}
\]

\[\text{aPL} \quad \text{antiphospholipid antibodies}
\]

\[\text{APS} \quad \text{antiphospholipid syndrome}
\]

\[\text{aV} \quad \text{antibodies to DM V}
\]

\[\text{awM} \quad \text{antibodies to WM}
\]

\[\text{CL} \quad \text{cardiolipin}
\]

\[\text{DM} \quad \text{deleted mutants}
\]

\[\text{PC} \quad \text{phosphatidylincholine}
\]

\[\text{PGE}_1 \quad \text{prostaglandin E}_1
\]

\[\text{PI} \quad \text{phosphatidylinositol}
\]

\[\text{PL} \quad \text{phospholipid}
\]

\[\text{PRP} \quad \text{platelet-rich plasma}
\]

\[\text{PS} \quad \text{phosphatidylserine}
\]

\[\text{WM} \quad \text{whole molecule (β2GPI)}
\]

References


Properties of anti-β2GPI antibodies


