Prevalence of β_2 -glycoprotein I antibody in systemic lupus erythematosus patients with β_2 -glycoprotein I dependent antiphospholipid antibodies

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Abstract

Objective—To investigate whether β_2 -glycoprotein I antibody (aGPI) exists in sera that are positive for antiphospholipid antibodies (aPL).

Methods—Thirty six patients with systemic lupus erythematosus (SLE) and 20 healthy volunteers were recruited. GPI independent and dependent aPL (anticardiolipin (aCL), antiphosphatidylserine, antiphosphatidylinositol, antiphosphatidic acid) were measured using a conventional enzyme linked immunosorbent assay (ELISA). aGPI was measured by an ELISA using a γ irradiated plate coated with purified GPI.

Results—Twenty of 36 SLE patients were positive for at least one of the GPI-dependent aPL. Five of these 20 were also positive for aGPI. Another five of the 20 showed low GPI dependency aCL antibodies; however, three of these patients required GPI for binding to phosphatidylserine, phosphatidylinositol, or both.

Conclusion—We confirmed the existence of aGPI in patients with true GPI dependent aPL. From these results, we recommend the use of a γ irradiated plate with GPI as antigen for the detection of true GPI dependent aPL or aGPI.

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Antiphospholipid antibody (aPL),^{1,2} sometimes detected in patients with systemic lupus erythematosus (SLE), antiphospholipid syndrome, or both, was initially thought to be an antibody to phospholipid (PL) itself. Subsequently, it was recognised as an antibody to PL/ β_2 -glycoprotein I (GPI),³ and is thought to be important in the aetiology of aPL related complications, such as arteriovenous thrombosis and recurrent abortion.^{1,2} Furthermore, it has been reported^{4–7} that aPL is an antibody to GPI itself. Matsuura *et al*⁸ confirmed that the majority of samples positive for anticardiolipin antibody (aCL) reflected reaction to GPI itself. Using a plate that was irradiated with an electron beam or cobalt-60, or both, they claimed that aCL was an antibody, not to cardiolipin, but to structurally modified GPI.

However, Gharavi *et al*⁹ were unable to detect anti-GPI antibody in samples positive for aCL even when they used a γ irradiated plate. Thus controversy remains as to whether the real antigen to aPL is PL-GPI or GPI.

Using a γ irradiated plate, we investigated whether aGPI was present in sera positive for at least one of four anionic phospholipid antibodies, including aCL.

Patients and methods

We studied 36 patients who fulfilled the diagnostic criteria for SLE¹⁰ and 20 healthy volunteers.

CONVENTIONAL ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

GPI independent and dependent antibodies to cardiolipin (aCL),¹¹ phosphatidylserine (aPS), phosphatidylinositol (aPI), and phosphatidic acid were measured by a method previously reported.¹² In brief, a plate coated with 30 μ l of cardiolipin, phosphatidylserine, phosphatidyl inositol, or phosphatidic acid (50 μ g/ml, Sigma, USA) was blocked with 1% fatty acid-free bovine serum albumin (BSA) (Sigma) and washed with 0.05% Tween 20-phosphate buffered saline (PBS). Fifty microlitres of GPI purified by affinity chromatography (confirmed to be free from phospholipid by sodium dodecyl sulphate polyacrylamide gel electrophoresis) in PBS was added for the measurement of GPI dependent aPL; 50 μ l of the same buffer alone was added for measurement of GPI independent aPL. Fifty microlitres of sample serum diluted in buffer (1:100) was added to duplicate wells. After two hours of incubation at room temperature, the plates were washed with the same buffer and 50 μ l of alkaline phosphatase (ALP)-conjugated antihuman IgG goat anti-serum (1:400) (Tago, USA) was added to each well. After incubation for one hour and washing, ALP activity was determined using p-nitrophenyl phosphate in diethanolamine buffer, by measurement of absorbance at 405 nm.

GPI independent and dependent aPL were judged to be present when the optical density (OD) of the sample exceeded the mean OD + 4SD of 20 healthy controls. The ratio

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of GPI dependency of the antibody was calculated using the formula:

$$\frac{\text{OD of GPI dependent aPL}}{\text{OD of GPI independent aPL}}$$

ANTI-GPI ANTIBODY (aGPI) ELISA

aGPI was measured using the method reported by Matsuura *et al.*⁸ with slight modifications. A sterile γ irradiated plate (Costar, USA) was coated with 50 μ l of purified GPI (20 μ g/ml) and left overnight at 4°C. The plates were washed with PBS containing 0.05% Tween 20 and test serum samples diluted 1:100 with PBS-BSA-Tween 20 were added. Subsequent procedures were as described above.

aGPI was considered positive when the ODs of the test sample exceeded the mean OD + 4SD of the 20 control samples. The specificity of aGPI in the sample was confirmed by a modified method of Matsuura *et al.*⁸ In brief, the serum shown positive for aGPI by the present method was mixed with PL liposome with or without GPI and subjected again to measurement for aGPI.

Results

At least one of four GPI independent aPLs was demonstrated to be present in 21 of 36 patients with SLE by the conventional ELISA (table). Twenty of those positive patients were also positive for GPI dependent aPL; their ratio of GPI dependent to GPI-independent aPL was in the range 0.8–9.0.

aGPI was positive in five of the 20 GPI dependent aPL group of SLE patients (patients 1–4, 11); the GPI dependency ratio of aPL in these five patients exceeded 1.5 (range 1.5–9.0). Three of the five patients (patients 1–3) had a previous history of thrombocytopenia, one (patient 3) had habitual abortion, one (patient 4) had thrombosis, and one (patient 3) had a biological false positive reaction to the serological test for syphilis; one (patient 11) had no previous history of aPL related complications (table). There was no obvious difference between groups with or without aGPI in the variety of their complications. None of the patients with a GPI dependency ratio less than 1.2 was positive for aGPI, even if they were positive for GPI dependent aPL by conventional ELISA.

Five of the 20 patients who tested positive for the presence of the GPI dependent group of phospholipid antibodies had a GPI dependency ratio less than 1.2 for binding to cardiolipin; in three of these five, the ratio was greater than 1.5 for aPS, aPI, or both.

None of the samples negative for aPL by conventional ELISA was positive for aGPI. aGPI in the serum mixed with PL liposome and GPI became negative, but no obvious change was observed using serum mixed with PL liposome without GPI; thus specificity for aGPI was confirmed in these patients.

Discussion

Matsuura *et al.*⁸ speculated that a polystyrene plate into which oxygen atoms had been

Clinical profiles and prevalence of β_2 -glycoprotein I-dependent antiphospholipid antibodies, anti β_2 -glycoprotein I antibody, or both, in patients with systemic lupus erythematosus

Patient	Sex	aPL				aGPI	GPI index	aPL related complications			
		aCL	aPS	aPI	aPA			TP	HA	THR	BFP
1	f	+	+	+	+	+	3.8	○	-	-	-
2	f	+	+	+	-	+	6.8	○	-	-	-
3	f	+	+	+	-	+	9.0	○	○	-	○
4	m	+	+	+	-	+	4.3	-	-	○	-
5	f	+	+	+	-	-	<1.2	-	○	-	○
6	f	+	-	-	-	-	<1.2	-	○	-	-
7	f	+	-	+	+	-	<1.2	-	○	-	-
8	f	-	+	+	-	-	<1.2	-	-	-	○
9	f	-	+	-	-	-	<1.2	-	○	-	-
10	f	+	+	+	+	-	<1.2	-	-	-	-
11	f	+	+	+	-	+	1.5	-	-	-	-
12	f	+	+	-	-	-	<1.2	-	-	-	-
13	f	+	-	-	+	-	<1.2	-	-	-	-
14	f	+	-	-	+	-	<1.2	-	-	-	-
15	m	+	-	-	-	-	<1.2	-	-	-	-
16	f	+	-	-	-	-	<1.2	-	-	-	-
17	f	-	+	-	-	-	<1.2	-	-	-	-
18	f	-	+	-	-	-	<1.2	-	-	-	-
19	f	-	-	+	-	-	<1.2	-	-	-	-
20	f	-	-	-	+	-	<1.2	-	-	-	-
21	f	-	+	-	-	-	<1.2	-	-	-	-
22	f	-	+	-	-	-	<1.2	-	○	○	-
23	m	-	-	-	-	-	<1.2	-	○	-	-
24	f	-	-	-	-	-	<1.2	○	-	-	-
25	f	-	-	-	-	-	<1.2	○	-	-	-
26	f	-	-	-	-	-	<1.2	○	-	-	-
27	f	-	-	-	-	-	<1.2	○	-	-	-
28	f	-	-	-	-	-	<1.2	-	-	○	-
29	f	-	-	-	-	-	<1.2	-	-	-	-
30	m	-	-	-	-	-	<1.2	-	-	-	-
31	f	-	-	-	-	-	<1.2	-	-	-	-
32	f	-	-	-	-	-	<1.2	-	-	-	-
33	f	-	-	-	-	-	<1.2	-	-	-	-
34	f	-	-	-	-	-	<1.2	-	-	-	-
35	f	-	-	-	-	-	<1.2	-	-	-	-
36	f	-	-	-	-	-	<1.2	-	-	-	-

aPL = Antiphospholipid antibodies; aCL = anticardiolipin antibody; aPS = antiphosphatidylserine antibody; aPI = antiphosphatidylinositol antibody; aPA = antiphosphatidic acid antibody; * β_2 -glycoprotein I (GPI)-independent aPL; aGPI = anti-GPI antibody; GPI index = ratio GPI dependent aPL:GPI independent aPL (see text); TP = thrombocytopenia; HA = habitual abortion; THR = arteriovenous thrombosis; BFP = biological false positive reaction to serological test for syphilis.

introduced by γ irradiation may provide a surface for attachment of GPI, thereby changing its conformational structure and leading to new epitope formation, introducing new antigenicity for aGPI measurement. According to this concept, cardiolipin used in a conventional ELISA would not act as an antigen for GPI dependent aCL, but would serve as a modifier of the plate surface for the attachment and structural modification of GPI.

We confirmed the existence of aGPI in five of 20 SLE patients positive for GPI dependent aPL as judged by conventional ELISA. However, there was no obvious difference between the groups with or without aGPI in terms of variety of associated complications. Further studies are required to confirm the clinical relevance of aGPI and establish if there is a greater incidence of clinical complication(s) related to the presence of aGPI/GPI dependent aPL.

It is noteworthy that the GPI dependency ratio was less than 1:2 in all of the patients without aGPI, even if they were positive for GPI dependent aPL by conventional ELISA. These results suggest that a considerable proportion of aPL which appears to be GPI dependent on the basis of conventional ELISA might be an antibody that recognised phospholipid alone as antigen, and it may be preferable to calculate the ratio of GPI dependency in order to confirm the presence of truly GPI dependent aPL when measuring aPL by conventional ELISA.

Our observation of within-patient differences in GPI dependency ratios for antibodies to individual phospholipids (aCL, aPS, aPI) suggests that anionic phospholipids have subtly different effects on the plate surface or GPI structure, causing the ratio of GPI-dependency of aPL to vary considerably in individual conventional ELISAs, even when the same serum is used.

Because Matsuura *et al*⁸ reported a high correlation between the ODs of GPI dependent aCL and those of aGPI, it may be assumed that all their sera positive for aCL were also positive for aGPI, though they did not refer to positivity in their paper. Our results suggest that some GPI dependent aPL or aGPI in the samples may not be detected by use of conventional aCL ELISA alone, but it is obviously impossible routinely to test all sera using a variety of phospholipids as antigen. As we were

able to confirm the existence of aGPI in SLE patients with aPL using the method reported by Matsuura *et al*,⁸ this method may be an appropriate and alternative routine approach to determining true GPI dependent aPL or aGPI. Moreover, the method may be substituted by conventional ELISA if the GPI dependency ratio we propose in the present study is used to calculate GPI dependency. Further studies based on larger samples are required to confirm the validity of this method.

Gharavi *et al*⁹ failed to confirm the existence of aGPI in aCL positive sera even though they used a γ irradiated plate. This discrepancy in results may reflect differences in the samples examined or the quality of plate used, but such explanation remains speculative.

On the basis of our findings, we recommend the use of a γ irradiated plate for the detection of GPI dependent aPL or aGPI.

- McNeil H P, Krilis S A. Antiphospholipid antibodies. *Aust NZ J Med* 1992; 21: 463-75.
- Triplett P A. Antiphospholipid antibodies and thrombosis. A consequence, coincidence, or cause? *Arch Pathol Lab Med* 1993; 117: 78-88.
- McNeil H P, Simpson R J, Chesterman C N, Krilis S A. Antiphospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation; β_2 -glycoprotein I (apolipoprotein H). *Proc Natl Acad Sci USA* 1990; 87: 4120-4.
- Galli M, Confurius P, Maassen C, *et al*. Anticardiolipin antibodies directed not to cardiolipin but to a plasma protein cofactor. *Lancet* 1990; 335: 1544-51.
- Arvieux J, Roussel B, Jacob M C, Colomb M G. Measurement of antiphospholipid antibodies by ELISA using β_2 -glycoprotein I as an antigen. *J Immunol Methods* 1991; 143: 223-9.
- Viard J P, Amoura Z, Bach J F. Association of anti- β_2 -glycoprotein I antibodies with lupus-type circulating anticoagulant and thrombosis in systemic lupus erythematosus. *Am J Med* 1992; 93: 181-6.
- Matsuura E, Igarashi Y, Fujimoto M, *et al*. Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. *J Immunol* 1992; 148: 3885-91.
- Matsuura E, Igarashi Y, Yasuda T, Triplett D, Koike T. Anticardiolipin antibodies recognize β_2 -glycoprotein I structure altered by interacting with an oxygen modified solid phase surface. *J Exp Med* 1994; 179: 457-62.
- Gharavi A E, Harris E N, Sammaritano L R, Perangeli S S, Wen J. Do patients with antiphospholipid syndrome have autoantibodies to β_2 -glycoprotein I? *J Lab Clin Med* 1993; 122: 426-31.
- Tan E M, Cohen A S, Fries J F, *et al*. Revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271-7.
- Matsuda J, Saitoh N, Gohchi K, Gotoh M, Tsukamoto M. Distinguishing β_2 -glycoprotein I dependent (systemic lupus erythematosus type) and independent (syphilis type) anticardiolipin antibody with Tween 20. *Br J Haematol* 1993; 85: 799-802.
- Matsuda J, Saitoh N, Gohchi K, Gotoh M, Tsukamoto M. Detection of β_2 -glycoprotein I-dependent antiphospholipid antibodies and anti- β_2 -glycoprotein I antibody in patients with systemic lupus erythematosus and in patients with syphilis. *Int Arch Allergy Immunol* 1994; 103: 239-44.