



Lupus anticoagulant

Mode of action

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Anti-phospholipid syndrome

A patient with:

1. thrombosis
recurrent pregnancy loss
&
2. lupus anticoagulant
anti-cardiolipin antibodies
anti- β_2 -glycoprotein I
antibodies

diagnosis

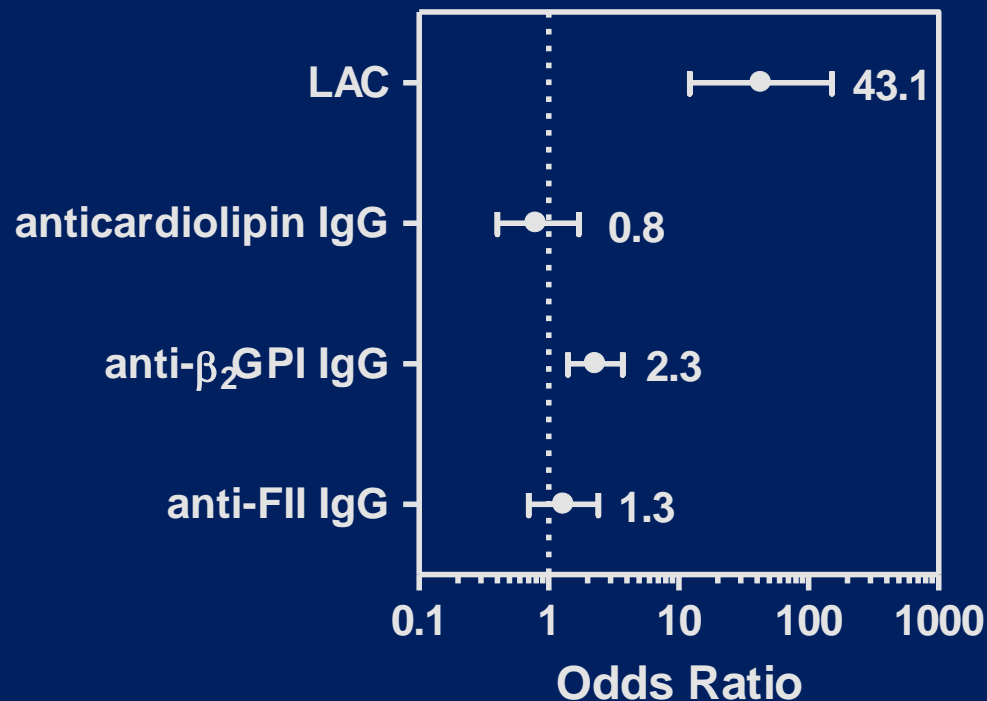
ANTI-
PHOSPHOLIPID
SYNDROME

Sapporo: Wilson et al. *Arthritis Rheum.* 1999; 42: 1309–11

Sydney: Miyakis et al. *J.Thromb. Haemostas.* 2006; 4: 295-306

Lupus anticoagulant: Assay of choice

Lupus anticoagulant is one of the strongest acquired risk factors for venous and arterial thrombosis.



RATIO study: Ischemic stroke in young women

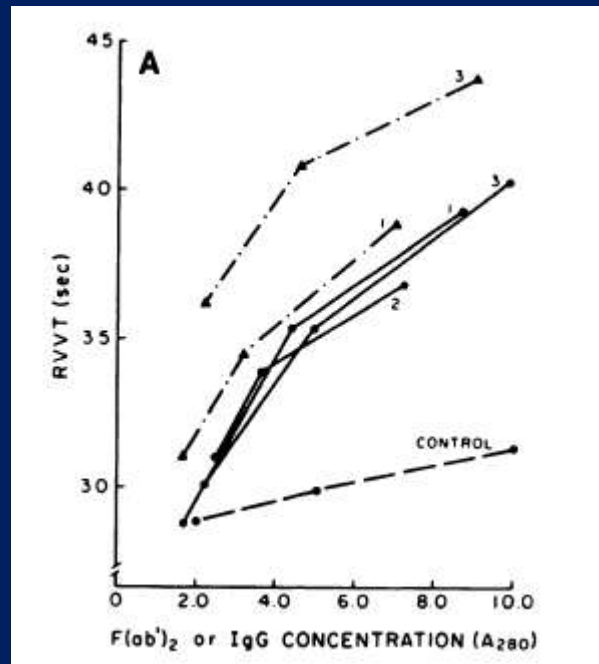


Lupus anticoagulant:
a prolongation of a clotting assay

What do we know about the mode of
action of lupus anticoagulant ?

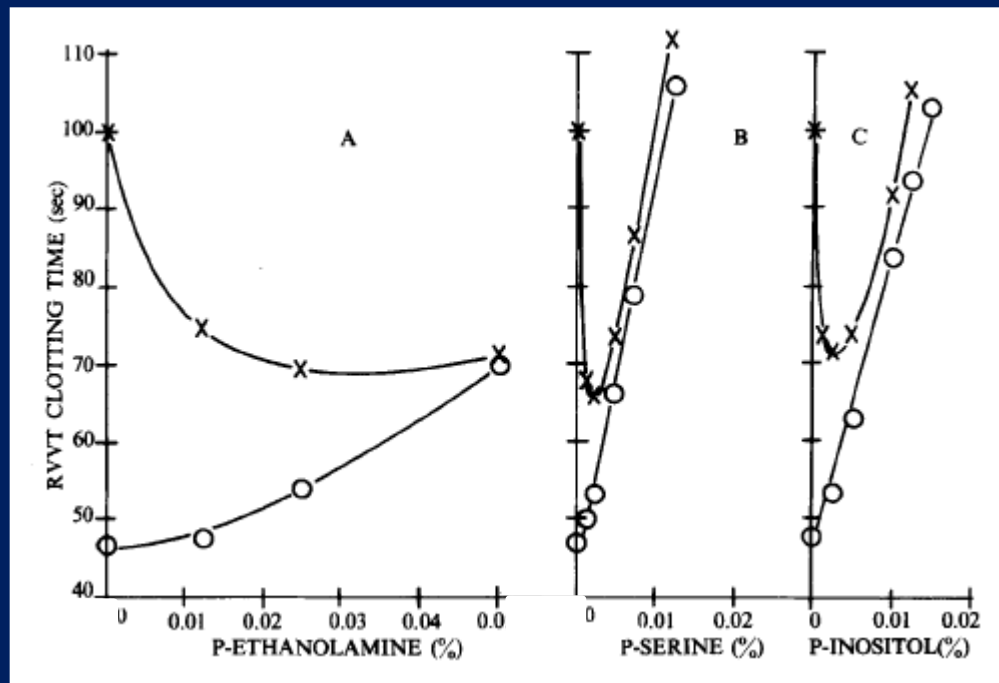
Lupus anticoagulant: what is known

Purified patient IgG induces prolongation of clotting when added to normal plasma → antibody (IgG, IgM, IgA)



Lupus anticoagulant: what is known

Inhibition by anionic phospholipids → **PL dependent**





Lupus anticoagulant: the assay

- Use of platelet poor plasma ($< 10^7$ /mL)
- Prolongation of a phospholipid-dependent clotting test.
Two assays: dRVVT and APTT.
- Demonstration of the presence of an inhibitor.
patient plasma mixed 1:1 with normal plasma.
- Demonstration that inhibitory activity is dependent on PL.
Increasing the concentration of phospholipids.

SSC guidelines:

Green et al. Thromb.Haemostas.1983; 49: 144-6

Exner et al. Thromb.Haemostas.1991; 65: 320-2

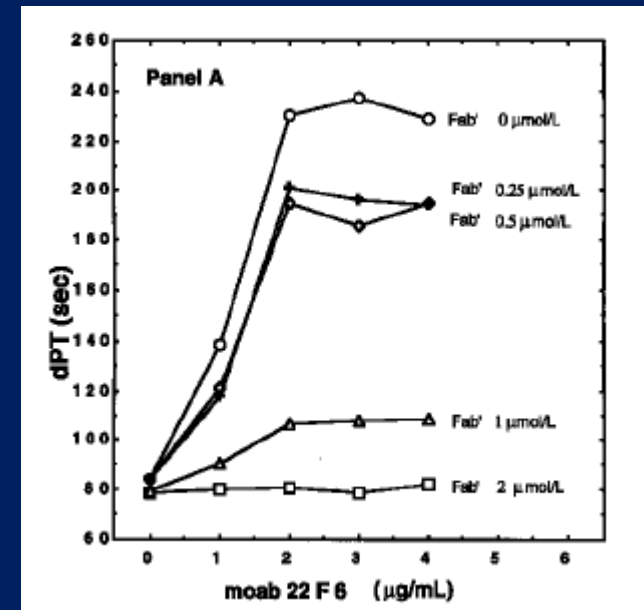
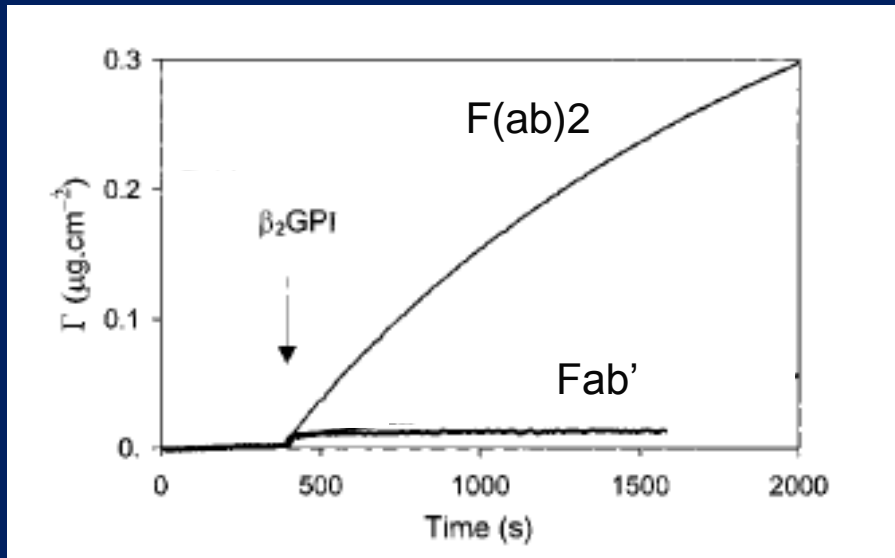
Brandt et al. Thromb.Haemostas.1995; 74: 1185

Pengo et al. J.Thromb.Haemostas.2009; 7: 1737-40

Lupus anticoagulant: what do we know more

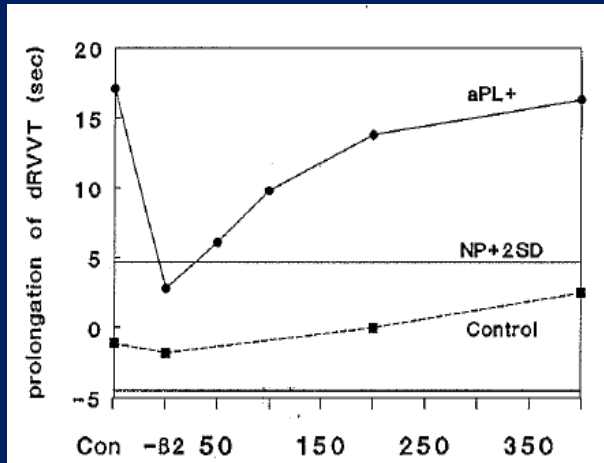


F(ab)2 fragments but not Fab fragments prolong clotting times → **dimerisation**

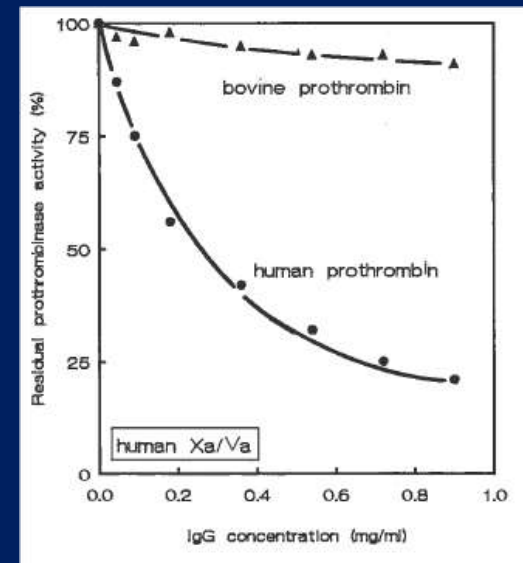


Lupus anticoagulant: what do we know more

- Phospholipid binding plasma proteins are necessary:
 - β_2 -glycoprotein I (3 μ M)
 - Prothrombin (1.4 μ M)
 - A combination of prothrombin and β_2 -glycoprotein I
 - Others?

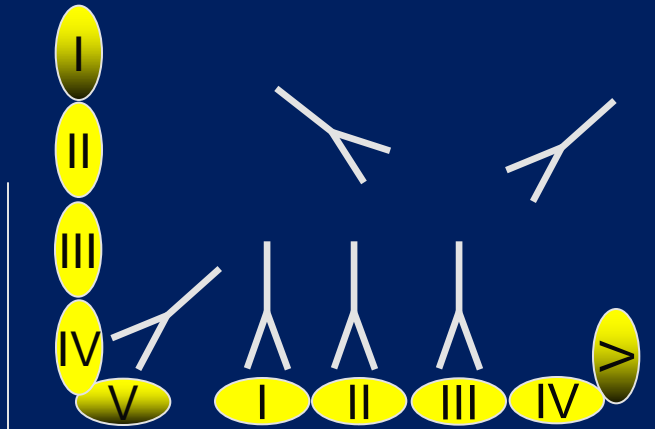


Oosting et al. TH 1992; 67:499-502

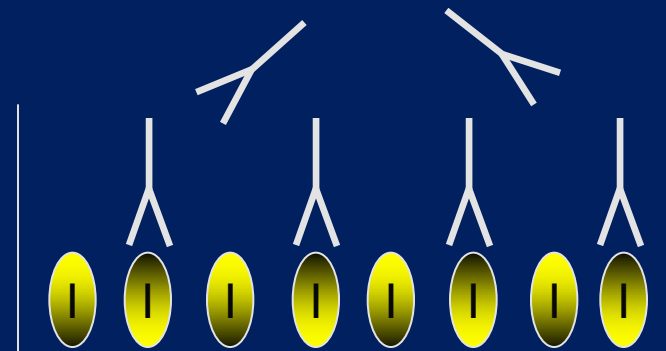


Bevers et al. TH 1991; 66:629-634

Antibodies against domain I of β_2 GPI



β_2 -glycoprotein I



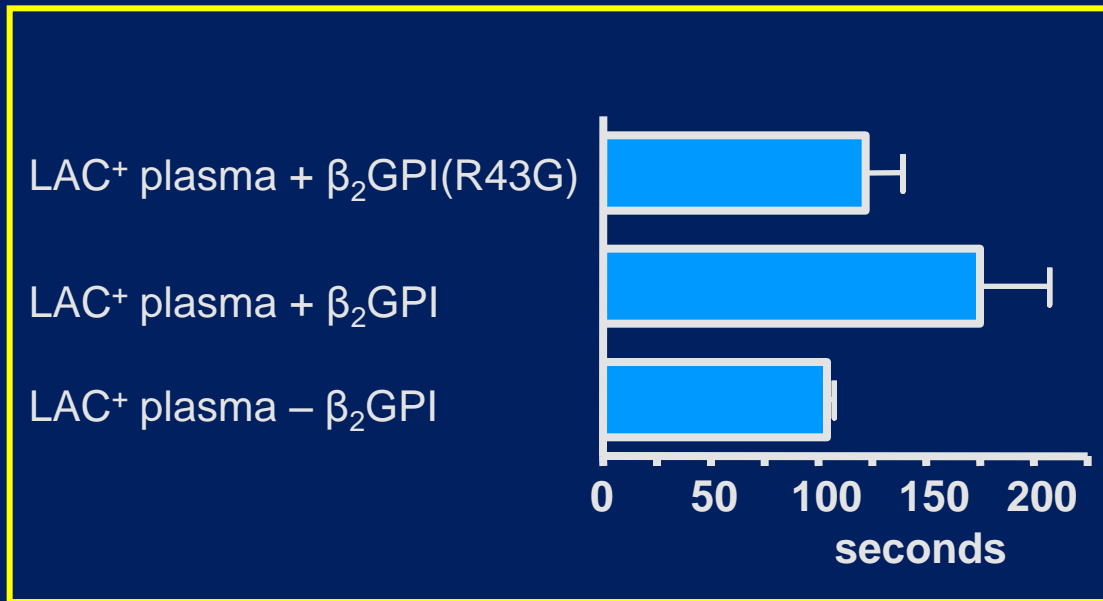
Domain I

The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study

B. DE LAAT,* V. PENGO,† I. PABINGER,‡ J. MUSIAL,§ A. E. VOSKUYL,¶ I. E. M. BULTINK,¶
A. RUFFATTI,** B. ROZMAN,†† T. KVEDER,†† P. DE MOERLOOSE,‡‡ F. BOEHLEN,§§ J. RAND,¶¶
Z. ULCOVA-GALLOVA,*** K. MERTENS* and P. G. DE GROOT†††

Anti-domain I antibodies: a high specificity but a low sensitivity

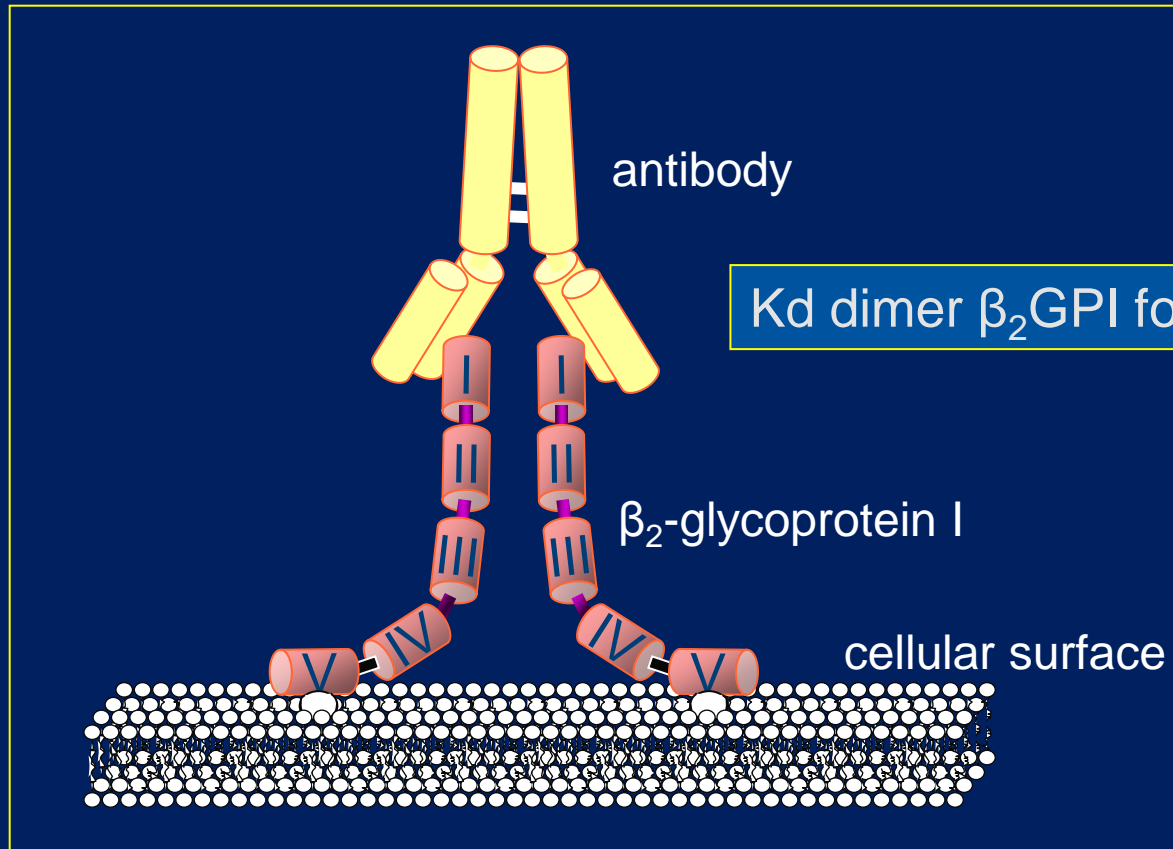
Domain I of β_2 Glycoprotein I & LAC



SLE patients

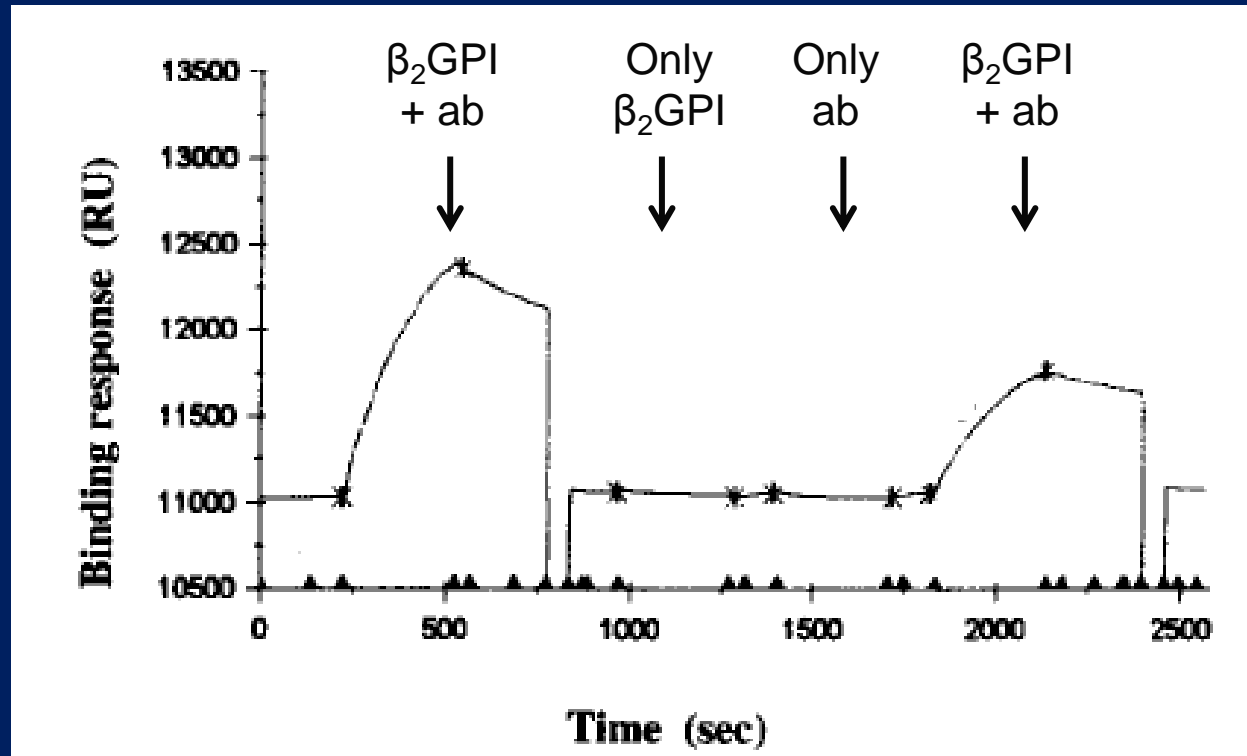
		β_2 GPI-dependent LAC	
		+	-
Domain I ELISA	+	23	7
	-	2	167

Cartoon to visualize mode of action



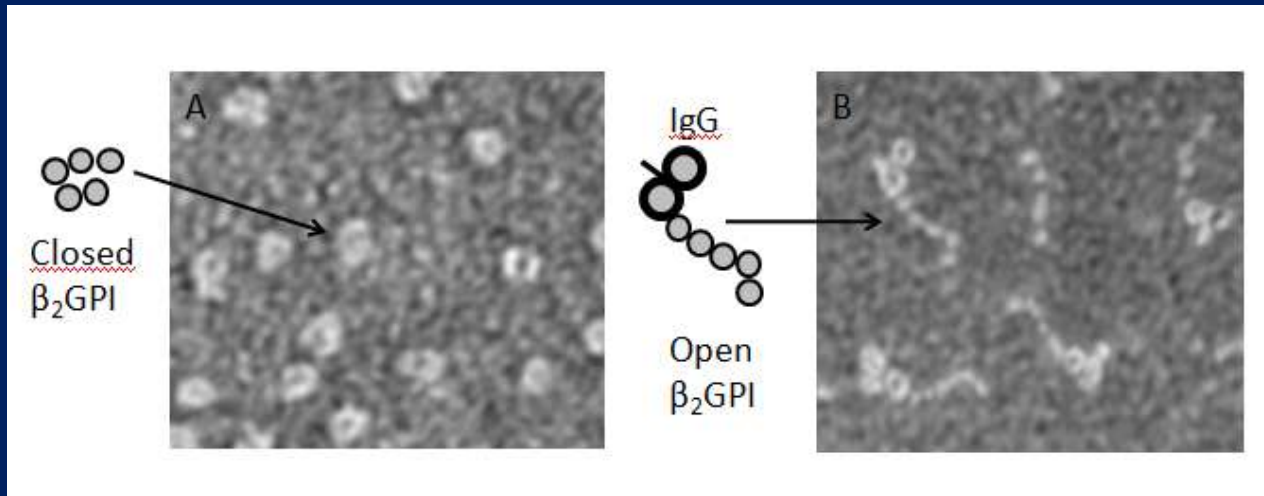
Dimerisation of β_2 -glycoprotein I increases its affinity for anionic phospholipids → competition with clotting factors

Binding of β_2 GPI to phospholipids



β_2 -glycoprotein I binds only in the presence of antibodies to anionic phospholipids

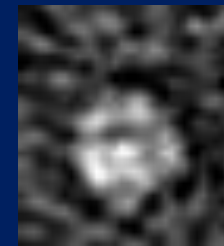
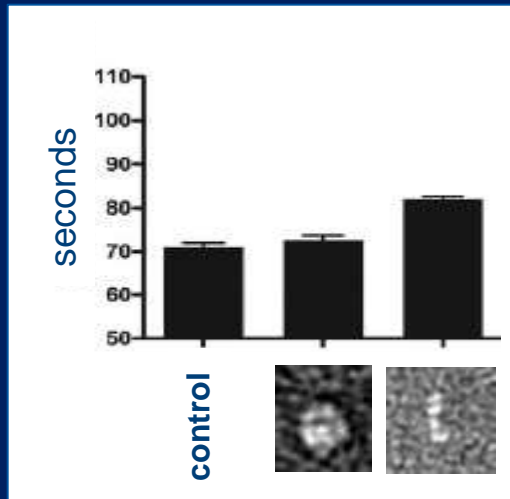
Conformation of β_2 -glycoprotein I



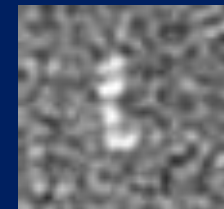
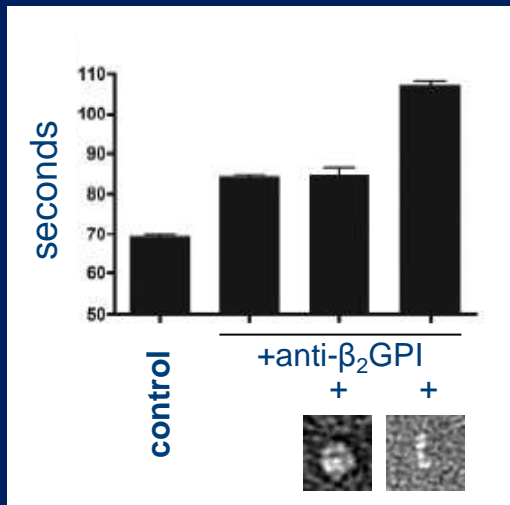
β_2 -glycoprotein I can exist in two conformation

- A circular conformation in plasma
- A stretched conformation after binding to PLs

Open β_2 -glycoprotein I inhibits coagulation

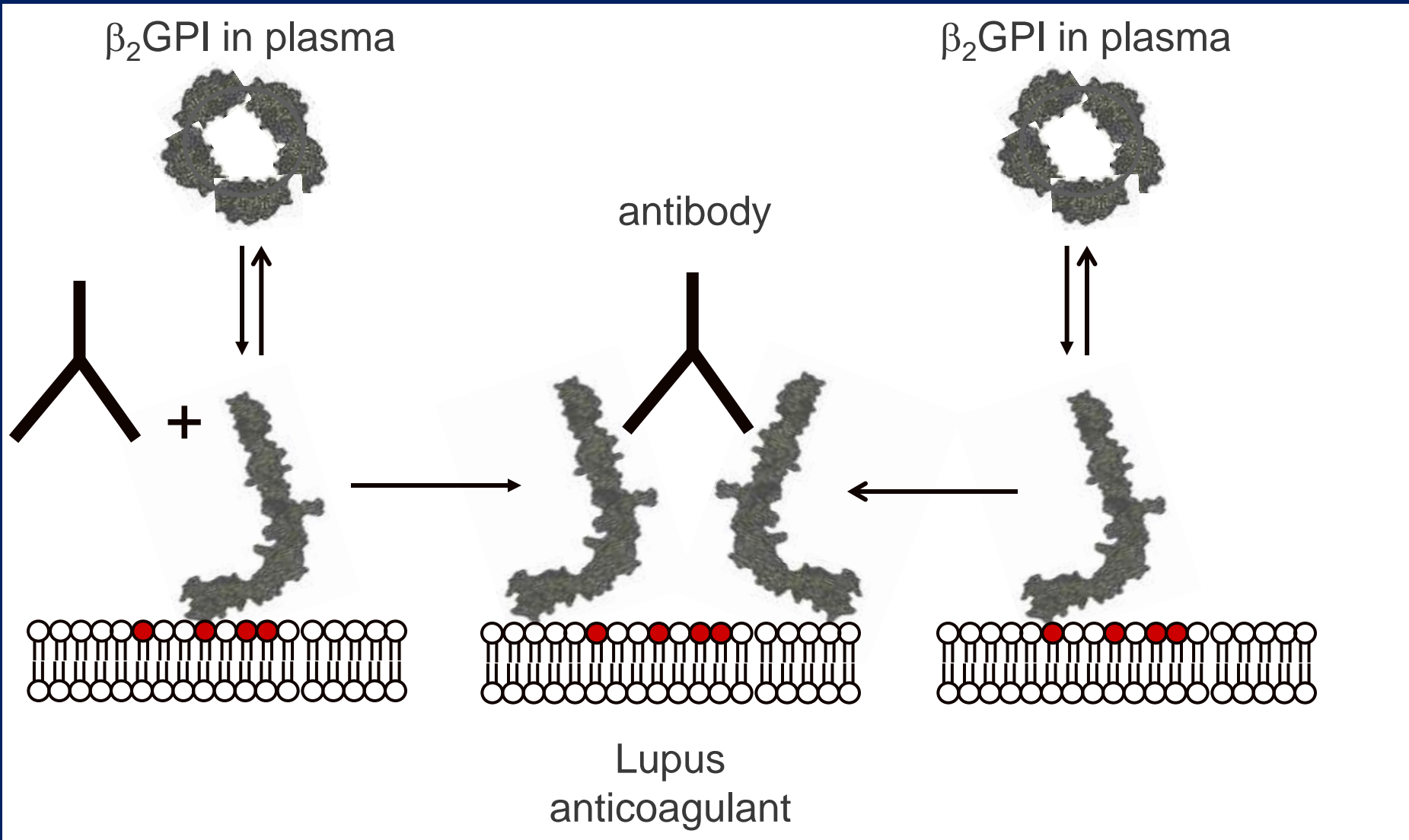


Circular β_2 GPI



Open β_2 GPI

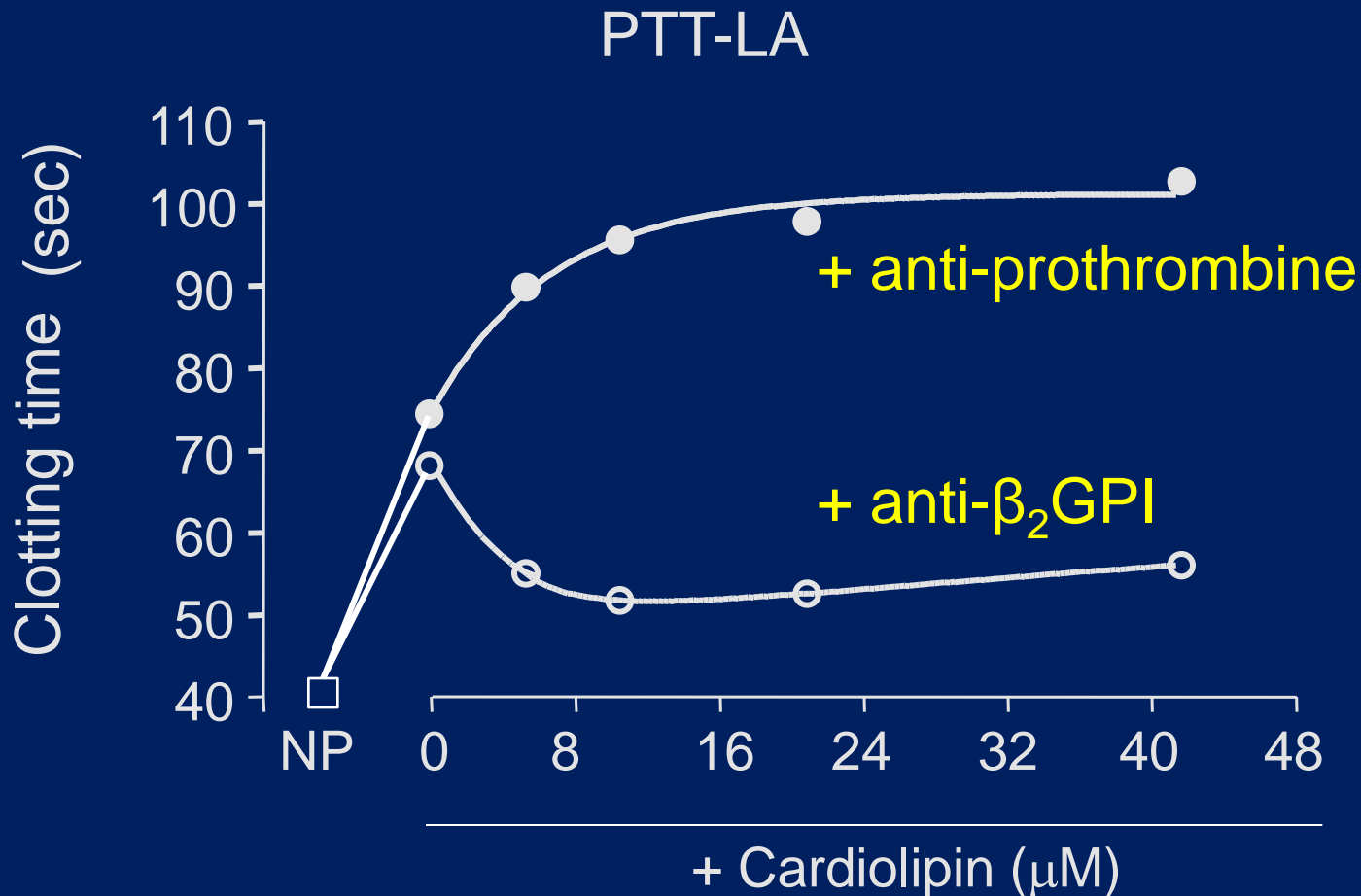
Model for lupus anticoagulant



Mode of action Lupus anticoagulant

- β_2 -Glycoprotein I
 - Binding to phospholipids → conformational change.
 - Antibody binding to domain I.
 - Dimerization by auto-antibodies → increased affinity.
 - Competition with clotting factors for available phospholipids .
- Prothrombin
 - Lower levels of prothrombin in plasma.
 - Antibodies recognize F1+2 part (?).
 - Binding of antibodies increases affinity for phospholipids.
 - Competition with clotting factors for available phospholipids .

Effect of cardiolipin on LAC activity





β_2 Glycoproteine I dependent LA

- ✓ 198 patients with SLE
 - *59 thrombotic complications*

- ✓ 'Classic' LA
 - *OR 10.2 (22.0 - 5.3)*

- ✓ β_2 -GPI dependent LA
 - *OR 42.3 (194.3 – 9.9)*

- ✓ β_2 GP independent LAC
 - *OR 1.6 (3.9 – 0.8)*

What do we know



- Lupus anticoagulant is the assay of choice to detect 'anti-phospholipid' antibodies
- Lupus anticoagulant depending on β_2 -glycoprotein I correlates better with the clinic than prothrombin dependent lupus anticoagulant
- Auto-antibodies directed against domain I induce lupus anticoagulant activity and seem to be pathological relevant antibodies
- Both a conformational change and dimerization of β_2 -glycoprotein I are essential to express lupus anticoagulant activity

Do we understand LA?



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Utrecht

Prolongation of clotting times is an indication for a bleeding tendency not for a thrombotic tendency.

Is Lupus anticoagulant an in vitro artifact?

Effect of purified anti- β_2 GPI auto-antibodies on thrombus size.



University Medical Center
Utrecht

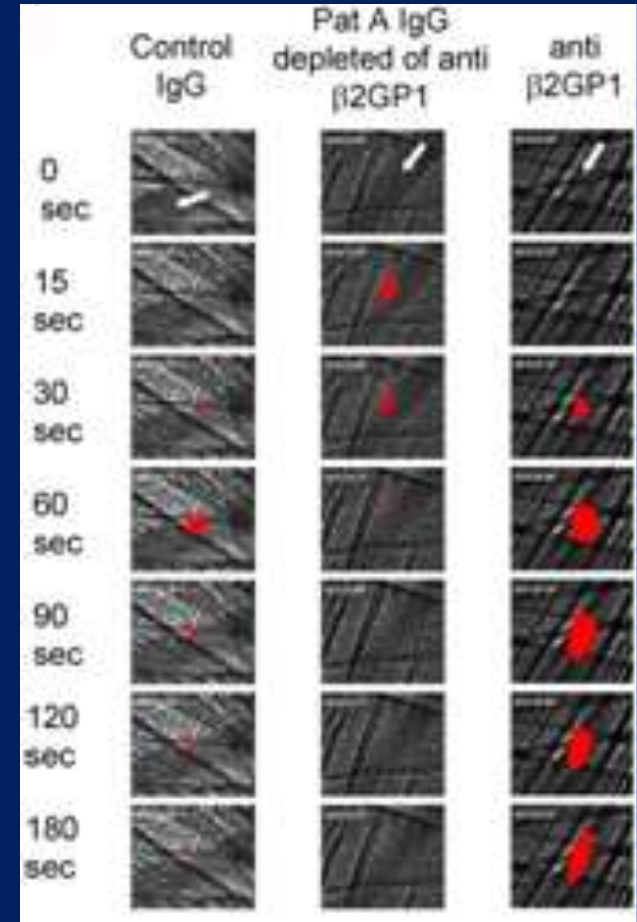
blood

2011 117: 3453-3459
Prepublished online January 18, 2011;
doi:10.1182/blood-2010-08-300715

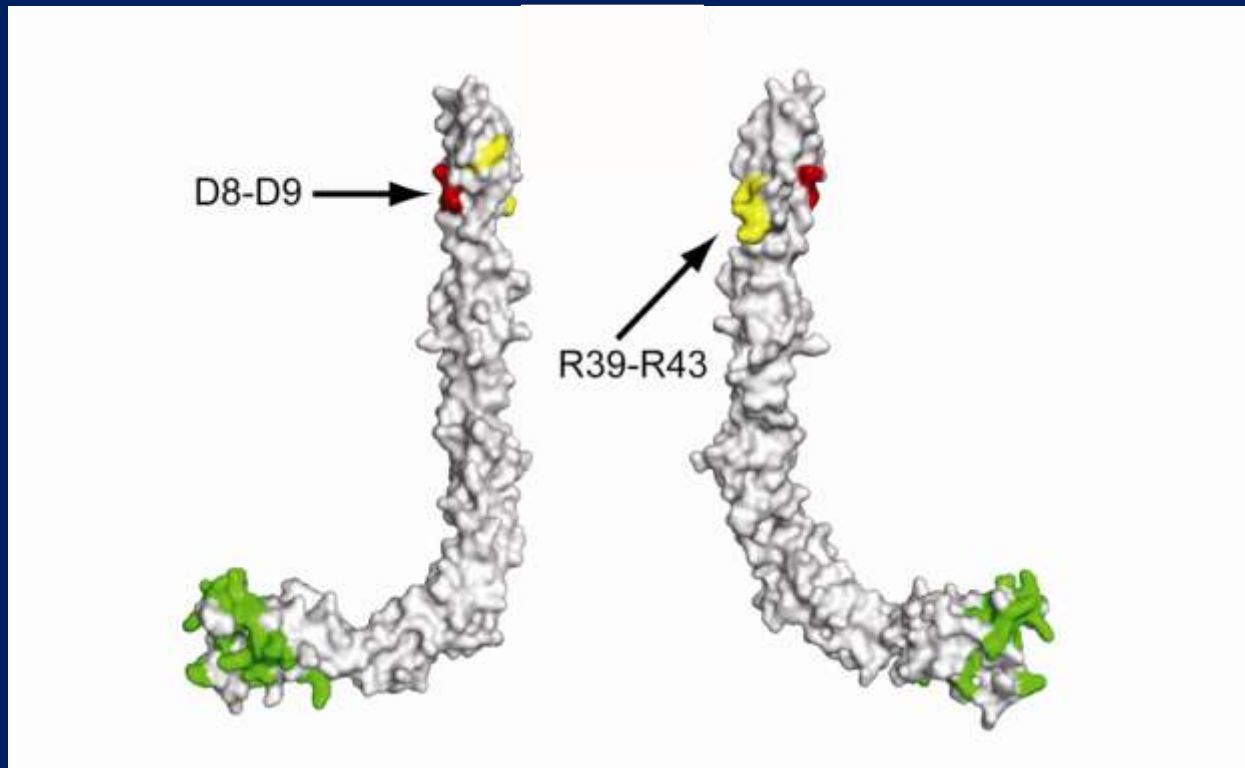
β_2 -glycoprotein-1 autoantibodies from patients with antiphospholipid syndrome are sufficient to potentiate arterial thrombus formation in a mouse model

Ariela Arad, Valerie Proulle, Richard A. Furie, Barbara C. Furie and Bruce Furie

Patient-derived auto-antibodies specific for β_2 -glycoprotein I enhanced dose-dependently a thrombotic response in a mouse model of APS



Antibody specificity



The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study

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Domain I as anti-thrombotic agent



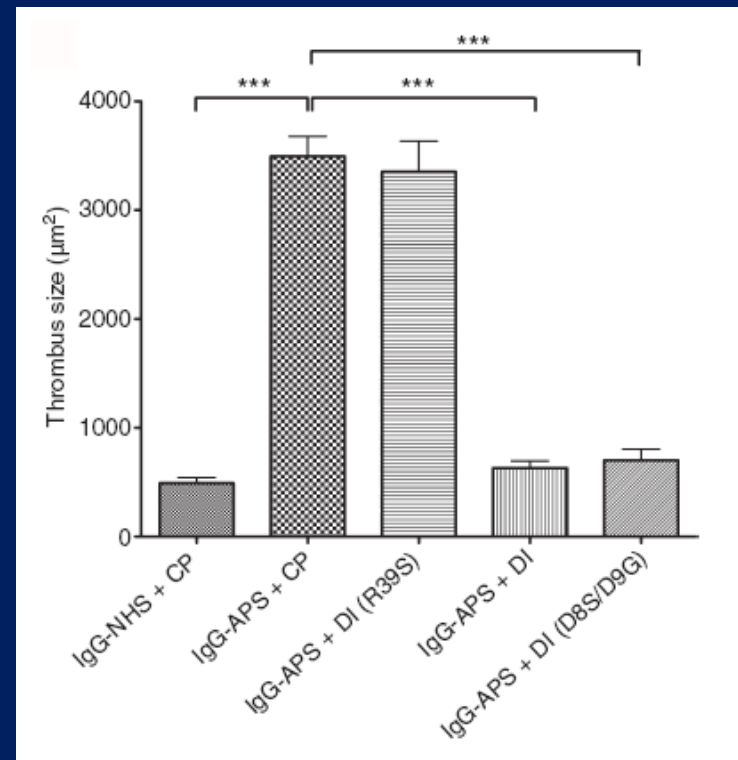
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Utrecht

J Thromb Haemost. 2009 May;7(5):833-42. Epub 2009 Feb 12.

In vivo inhibition of antiphospholipid antibody-induced pathogenicity utilizing the antigenic target peptide domain I of beta2-glycoprotein I: proof of concept.

Ioannou Y, Romay-Penabad Z, Pericleous C, Giles I, Papalardo E, Vargas G, Shilagard T, Latchman DS, Isenberg DA, Rahman A, Pierangeli S.

Domain I of β_2 -glycoprotein I can inhibit the increased thrombotic response induced by patient IgG in a mouse model of APS.



Conclusion

Anti- β_2 -glycoprotein I auto-antibodies expressing lupus anticoagulant activity seem to be responsible for the thrombotic complications in patients with APS.

Why do these antibodies cause a prolongation of clotting assays?

Does β_2 -glycoprotein I bind to phospholipids?

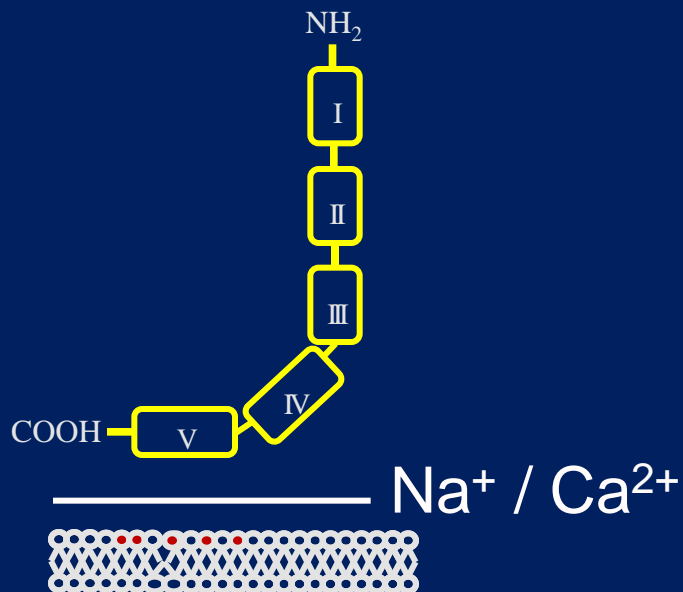


Biochemistry 1996, 35, 13833–13842

Role of Divalency in the High-Affinity Binding of Anticardiolipin Antibody– β_2 -Glycoprotein I Complexes to Lipid Membranes

George M. Willems,^{*,†} Marie P. Janssen,[†] Maurice M. A. L. Pelsers,[†] Paul Comfurius,[†] Monica Galli,[§] Robert F. A. Zwaal,[†] and Edouard M. Bevers[†]

in affinity. At physiologic conditions (10 mol % PS, 120 mM NaCl, and 3 mM CaCl₂), a K_d of 14 μ M was observed. Binding constants were insensitive to the chemical composition of the negatively charged



Na⁺ and Ca²⁺ inhibit the binding of β_2 -glycoprotein I to anionic phospholipids.

Lupus anticoagulant: artifact induced by citrate?

blood

1999 94: 3814-3819

Procoagulant Effect of Anti- β 2-Glycoprotein I Antibodies With Lupus Anticoagulant Activity

V. Pengo, T. Brocco, A. Biasiolo, P. Rampazzo, P. Carraro and R. Zamarchi

with PL at the beginning of the assay. In fact, modification of the standard diluted Russell viper venom time (dRVVT) test by adding calcium ions together with PL resulted in a loss of $\alpha\beta$ 2-GPI anticoagulant activity. The procoagulant effect was

Detection of the presence of lupus anticoagulant needs a pre-incubation step in the absence of Ca^{2+} .

Conclusions

Lupus anticoagulant is an auto-antibody that prolonged clotting times in routinely used clotting assays by competing with the clotting factors for the available anionic phospholipids

Its activity needs the presence of β_2 -Glycoprotein I or prothrombin but all the present evidence points to auto-antibodies against domain I of β_2 -Glycoprotein I as the culprits.

Lupus anticoagulant depends on the set-up of our assay systems, there is no evidence that lupus anticoagulant prolonged clotting in vivo.

Lupus anticoagulant is a surrogate biomarker for thrombosis.