

## BINDING OF $\beta$ 2GP1 TO VARIOUS PHOSPHOLIPID MIXTURES IS CALCIUM DEPENDENT AND IS DRAMATICALLY ENHANCED BY ANTIBODIES: UNDERSTANDING THE ANTICOAGULANT PARADOX

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### INTRODUCTION

Usually Anticardiolipin/Antiphospholipid Antibodies (ACA/APA) are measured using anionic phospholipids (Cardiolipin or Phosphatidic Acid) coated plates. The cofactor protein required for the binding of antibodies is brought by the saturation serum, the diluent or the tested plasma itself. Assays are usually performed in the absence of calcium. Assay conditions are then far away from physiological environment. We analysed binding of  $\beta$ 2GP1 to various phospholipid mixtures, without or with calcium, and how this binding is affected by presence of specific antibodies to  $\beta$ 2GP1, in order to understand how pathological mechanisms occur in vivo, and in order to search a link between the anticoagulant effect of antibodies associated with LA activity and their reactivity in ELISA.

### MATERIALS

- Highly purified and functional  $\beta$ 2GP1, non denaturated
- Various phospholipids (Phosphatidyl Serine: PS; Phosphatidyl Ethanolamine: PE; Phosphatidyl Choline: PC; cholesterol: Chl; Phosphatidic Acid: PA, all from Sigma).
- Anti  $\beta$ 2GP1 antibodies labelled with peroxidase (affinity purified rabbit polyclonal antibodies).
- Anti IgG Peroxidase conjugate.
- TMB substrate.
- Tris (0.05M), NaCl (0.15M), BSA (1%) at pH 7.50, without or with calcium (2.5mM).

### METHODS

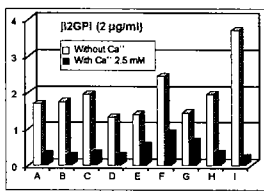
Phospholipids (PC, PS, PE, PA) or phospholipid mixtures were coated onto micro-ELISA plates (Nunc), then saturated with BSA and PEG: no phospholipid binding protein is present on the solid phase.

#### Phospholipid mixtures tested (%)

	PC	PE	PS	Chl	PA
A	100				
B	87.5	12.5			
C	50	37.5	12.5		
D	67				33
E	33				67
F			100		
G	100				
H	25	18	7	60	
I					100

### RESULTS

#### Binding of $\beta$ 2GP1 to PLPs without or with calcium

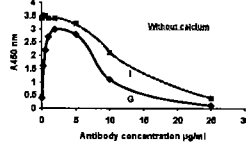


Presence of calcium (2.5 mM) strongly decreases binding of  $\beta$ GP1 to phospholipids and changes its phospholipid affinity pattern.

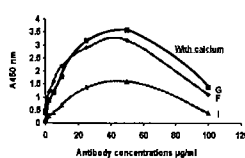
#### Dose dependent effect of antibodies on the binding of $\beta$ 2GP1 to PLPs

#### Anti- $\beta$ 2GP1 antibodies exhibit LA activity in clotting assays.

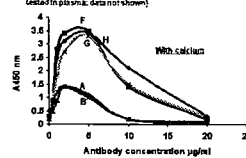
Binding of  $\beta$ 2GP1 (1 µg/ml) to high (F) or Low (E) PIP (without calcium) in presence of increasing concentrations of antibodies (same curve shape for bound antibodies: "order curve" when tested in plasma; data not shown)



Binding of  $\beta$ 2GP1 (1 µg/ml) to low affinity PLPs (A, B, C, D, E) in presence of calcium at different concentrations of antibodies (same curve shape for bound antibodies; "order curve" when tested in plasma; data not shown)



Binding of  $\beta$ 2GP1 (1 µg/ml) to low affinity PLPs in presence of calcium at different concentrations of antibodies (same curve shape for bound antibodies; "order curve" when tested in plasma; data not shown)



#### PROTOCOL 1

- Diluent with or without calcium (2.5 mM), supplemented with variable amounts of  $\beta$ 2GP1 is incubated with phospholipids.
- Following washing, an anti- $\beta$ 2GP1 coupled with peroxidase is added, and it allows measuring the amount of  $\beta$ 2GP1 bound to phospholipid surfaces.
- TMB is used for colour development.

#### PROTOCOL 2

- Diluent, contains a constant  $\beta$ 2GP1 concentration (with or without calcium) and is supplemented with variable amounts of polyclonal antibodies to  $\beta$ 2GP1.
- The amount of  $\beta$ 2GP1 bound is measured with an anti- $\beta$ 2GP1-peroxidase conjugate, and the amount of antibody bound is evaluated with an anti-IgG-peroxidase conjugate.

### CONCLUSIONS

- Binding of  $\beta$ 2GP1 to the various phospholipids is dependent on the calcium presence. Binding patterns differ greatly whether calcium is absent or present at physiological concentrations.
- Presence of anti- $\beta$ 2GP1 antibodies dramatically enhance binding of  $\beta$ 2GP1, as well as its antibodies, to low affinity phospholipids, especially in presence of calcium. There is a hook effect when excess of antibodies is present.
- The highest amount of  $\beta$ 2GP1 and antibodies bound are dependent on the phospholipid composition,  $\beta$ 2GP1 concentration and antibody concentration.
- Particular phospholipid structures (as can occur in some pathophysiological conditions) can be exposed and bind high amounts of  $\beta$ 2GP1 antibody complexes in patients with antibodies.
- This observation suggests a mechanism for the pathological effects of antibodies in vivo: they enhance binding of  $\beta$ 2GP1 to low affinity phospholipids, onto they form antibody-protein complexes, targeting the immune system harmful effect.

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