

## **ANTIPHOSPHATIDYL SERINE ANTIBODIES REQUIRE B<sub>2</sub> GLYCOPROTEIN I AS COFACTOR IN ELISA.**

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The role of B<sub>2</sub> glycoprotein I (B<sub>2</sub>GPI) as a cofactor in the binding of anticardiolipin (aCL) antibodies in ELISA has been extensively studied. Autoimmune aCL antibodies may be distinguished from non-autoimmune by their higher requirement for B<sub>2</sub>GPI. Phosphatidyl serine (PS) is also a negatively-charged phospholipid regarded as more physiologic than CL. This should make the detection of antiphosphatidyl serine (aPS) antibodies more clinically relevant than aCL antibodies in the diagnosis of the Antiphospholipid Syndrome. However, the role of B<sub>2</sub>GPI in the binding of aPS antibodies by ELISA has not been fully characterized. We investigated the requirement of B<sub>2</sub>GPI in an ELISA system developed to detect IgG aPS antibodies in human serum. Serial dilutions of selected aPS positive samples showed varying degrees of dependency when tested in the presence or absence of bovine serum (BS) in the sample diluent as the only source of cofactor. When purified human B<sub>2</sub>GPI was coated with PS, the absence or presence of BS did not affect the results, supporting the role of B<sub>2</sub>GPI in this interaction. Antibodies to B<sub>2</sub>GPI were not detected in these serum samples and a syphilis IgG aCL positive sample did not react with PS. We also used this system to test the role of B<sub>2</sub>GPI on IgM and IgA aPS antibodies. These isotypes required B<sub>2</sub>GPI but their interaction with PS appears to be more dependent on human B<sub>2</sub>GPI, while IgG aPS may use either human or bovine B<sub>2</sub>GPI as cofactor. To further evaluate the specific role of B<sub>2</sub>GPI as cofactor for IgG aPS antibodies, we coated human prothrombin or methylated BSA (as control) with PS at the same concentration used for B<sub>2</sub>GPI. No cofactor effect was seen in this system with these two proteins. These results indicate that autoimmune IgG aPS antibodies require B<sub>2</sub>GPI as cofactor and that IgM and IgA aPS also require this cofactor, but the nature of the B<sub>2</sub>GPI/PS interaction appears to be different depending on the origin of the cofactor. Higher binding affinity of IgG aPS antibodies may account for their interaction with both human and bovine cofactor. In addition, the lack of cofactor activity with other protein molecules (i.e. human prothrombin) in this system supports the concept that the specificity of aPS antibodies is directed mainly toward the B<sub>2</sub>GPI portion of the B<sub>2</sub>GPI/PS complex.

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