



ANTIPHOSPHOLIPID ANTIBODIES : MECHANISMS OF THROMBOSIS

The association between autoantibodies with specificity toward negatively-charged phospholipids and the clinical manifestations of the antiphospholipid syndrome has been well established and is now widely recognized. However, the exact mechanism(s) by which pathogenic antiphospholipid antibodies induce thrombosis *in vivo* is still not fully understood and is the subject of intense research. Recent advances, mostly on the nature of the antigens targeted by antiphospholipid antibodies, have been valuable not only to confirm their role as serologic markers for the antiphospholipid syndrome but also to develop hypotheses on how these antibodies may produce thrombosis. Two significant findings that have provided some clarification to this field are: a) the active role of phospholipids in the coagulation cascade, including externalization in the membranes of endothelial cells and platelets following injury, and b) the role of a serum cofactor (i.e. B2GPI) in the antibody binding activity. These findings are incorporated in most hypotheses on the mechanism of thrombosis in the antiphospholipid syndrome.

One model has been proposed to explain both the production of antiphospholipid antibodies and their role in thrombosis. It assumes an initial and perhaps recurrent injury of the vascular endothelium which results in the exposure of membrane phospholipids, such as phosphatidylserine (PS). These externalized phospholipids which are normally found in the inner layer of the lipid membrane become part of a pro-coagulant surface. In addition to activating coagulation factors, the exposed phospholipid is now able to bind circulating protein cofactors such as B2GPI or prothrombin. This binding will create a phospholipid-cofactor complex which will produce a neo-epitope capable of generating an immune response. The antibodies produced would be directed toward specific neo-epitopes produce by the type of phospholipid-cofactor complex formed i.e. PS-B2GPI or PS-prothrombin. Individuals with repeated or more extensive injuries of the vascular

endothelium will produce more antiphospholipid antibodies. Subsequent vascular injury in an individual with high serum antibody levels will most likely produce thrombosis. Thus, the presence of high levels of antiphospholipid antibodies in "healthy" individuals confers an increased risk for thrombosis following vascular injury.

The antibody binding to the phospholipid-cofactor complex, by itself, however, may not fully explain the activation of pro-coagulant mechanisms and thrombosis. It has been further postulated that antiphospholipid antibodies will concentrate on the cell surface and bind to immunoglobulin Fc receptors now exposed on the affected cell. This binding will promote certain metabolic modifications or biochemical changes on endothelial cells and platelets, such as the release of ADP and serotonin, the inhibition of prostacyclin, an increase of thromboxane, platelet activating factor, tissue factor, and adhesion molecules, etc. All of these changes promote thrombosis. This model is based on similar observations and mechanisms currently accepted to explain the clinical manifestations in patients with heparin-induced thrombocytopenia.

Antiphospholipid antibodies may also act *in vivo* at different levels of the coagulation cascade to promote thrombosis and to generate the other clinical manifestations of the antiphospholipid syndrome such as fetal loss and thrombocytopenia. Several studies have been published and mechanisms of action proposed. These antibodies may affect the fibrinolytic pathway by inhibiting the activation of Protein C, antithrombin III activity, Factor XII-dependent fibrinolysis or by increasing PAI-1. In addition, antiphospholipid antibodies may also inhibit the anticoagulant activity of circulating B2GPI. Of these mechanisms, the inhibition of the Protein C and Protein S pathways including the thrombomodulin receptor, has been implicated as clinically significant in the development of thrombosis in patients with the antiphospholipid syndrome.

THE READER RESPONSE

Q. Due to an increase in our aCL IgG/IgM test volume, we are now ordering 288-well (3-plate) aCL kits, rather than 96-well kits. One bottle of 33XPBS concentrate is supplied with the 288-well kit, with the instructions to dilute 30mL of concentrate to 1 liter. Will this be enough washing buffer for all three plates?

A. Unfortunately, the label on the 33XPBS wash buffer concentrate in the 288-well kit has generated some confusion. Although the bottle label states to dilute 30mL concentrate to 1 liter, the bottle contains 60mL 33XPBS concentrate, or enough for 2 liters of working wash buffer solution. A few cases have been reported where the entire bottle was emptied into a 1 liter container and subsequently filled to volume with distilled water. Decreased absorbance readings were recorded for both IgG and IgM assays when the more concentrated (2X) washing solution was used for plate washing. To eliminate further confusion, we are revising the bottle label on the wash buffer concentrate and the instructions in the package insert for preparing working wash solution. In the meantime, please be sure to measure 30 mL of wash concentrate (33XPBS) before diluting to 1 liter. Two liters of working washing solution should be sufficient for a 288-well kit.

Q. Our laboratory is planning to offer a panel for anti-phospholipid antibodies to include both REAADS Anti-Phosphatidylserine (aPS) and Anti-Cardiolipin (aCL) assays along with various tests for lupus anticoagulant. How should the aPS assay be billed? Is there a separate CPT code for aPS?

A. Currently, the same CPT billing code, 86147, must be used for both aPS and aCL tests. An application for a new billing code for aPS has been submitted to the AMA for review, however, the process is not expected to be completed before June, 1997.

Additional questions or requests for references regarding the information or opinions presented in this newsletter, current applications and clinical significance of antiphospholipid or autoimmune antibodies detected by ELISA may be directed to our customer service/ technical support staff at REAADS Medical Products, Inc.

READER PRODUCT FEATURE

REAADS Anti-Phosphatidylserine IgG/IgM ELISA Test Kit

For *in vitro* Diagnostic Use

Assay format -	96-well microtiter plate (8 x 12 strips) with breakaway wells
Antigen substrate -	Brain phosphatidylserine
Conjugate -	Horseradish peroxidase (HRP) / goat anti-human IgG/IgM
Chromogenic substrate -	TMB (single component)
Sample dilution -	1:50
Incubations	
Sample -	15 min @ room temperature
Conjugate -	15 min @ room temperature
Substrate -	10 min @ room temperature
Stopping solution -	2.5 N Sulfuric acid
Wavelength -	450 nm
Clinical specificity -	IgG: 96%; IgM: 96%
Clinical sensitivity -	unselected SLE: IgG 32%; IgM 7.5% SLE with thrombosis: IgG 54%; IgM 15%

READER ANNOUNCEMENTS

Merry Christmas!

Our best wishes to you and your families this Holiday Season from all of us at REAADS! We thank you for your business, and look forward to your continued support in the New Year.

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REAADS will be operating on a reduced schedule during the holidays between Thursday, December 19 Thursday, January 2. Please plan ahead to assure an adequate supply of kits during the holidays. For technical service or an emergency delivery during this period, please call and leave a detailed message with your phone number at (800) 729-5661, or (303) 457-4345 for International customers. One of our Customer Service Representatives will return your call as soon as possible.

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