◆ Special Lecture ◆

Antiphospholipid-Protein Antibodies

—A Paradigm for Antibody-Mediated Thrombosis—

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Antiphospholipid-protein antibodies (APA) are a family of immunoglobulins which recognize a number of plasma proteins that bind to activated phospholipid membranes. Among these plasma proteins are: $\beta 2$ Glycoprotein I ($\beta 2$ GPI), prothrombin, Annexin V, and other vitamin K dependent proteins (e.g., factor X). APA have been associated with venous and arterial events as well as recurrent spontaneous abortion (RSA). It is estimated that 8 to 14% of venous thrombosis is secondary to the presence of APA. APA are the most common cause of acquired thrombophilia. The concept of antibody-mediated thrombosis is now widely accepted.

The laboratory diagnosis of APA requires the use of coagulation-based tests to identify lupus anticoagulants (LA) and ELISA assays to identify anticardiolipin antibodies (ACA). The importance of testing for antibodies to $\beta 2$ GPI is now well recognized. Antibodies to $\beta 2$ GPI are more specific in identification of patients at risk for thrombosis.

Key words: anticardiolipin, lupus anticoagulant, thrombosis

Introduction

Antiphospholipid-protein antibodies (APA) are a family of immunoglobulins which have been defined by *in vitro* laboratory tests (Table 1)^{1,2}. The Immunoglobulins may be IgG, IgM, or IgA isotypes. Individual patients often have a mixture of isotypes. Recently, the concept of these antibodies having specificity for phospholipids has been challenged. The work of McNeil et al., Galli et al., and Matsuura et al. demonstrated that anticardiolipin antibodies (ACA) are not directed against cardiolipin but instead appear to recognize a plasma protein: $\beta 2$ Glycoprotein I ($\beta 2$ GPI)^{3,4,5}. Following these initial observations, Bevers and colleagues

found lupus anticoagulants (LA) recognize human prothrombin rather than phospholipids⁶. Both ACA and LA recognize specific plasma proteins when they are bound to activated phospholipid (PL) membranes *in vivo* (e.g., perturbed endothelium, platelets, monocytes). Presumably, these PL-binding proteins when reacting with

Table 1 Antiphospholipid Antibodies (APA)

I)	Lupus Anticoagulants
II)	Anticardiolipin Antibodies
III)	Reagin
IV)	Specific Antibodies to Anionic Phospholipids Antiphosphatidylserine Antiphosphatidic Acid Antiphosphatidylinositol
V)	Specific Antibodies to Neutral Phospholipids Antiphosphatidylethanolamine

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Table 2 APA Classification

I) Autoimmune
a) Primary
Do not fulfill criteria for systemic lupus erythematosus
b) Secondary
Systemic lupus erythematosus
Other connective tissue diseases
c) Drug induced
Procainamide
Quinine
Quinidine
alpha-interferon
II) Alloimmune
a) Infections
Viral
Bacterial
Protozoal
Fungal
b) Malignancies
Hairy cell leukemia
Lymphoproliferative

activated PL membranes expose neoepitopes. The subsequent immunologic response to exposure of the neoepitopes results in LA and ACA formation. *In vitro* test systems using "high sensitivity" microtiter plates relay on the same phenomenon when testing for antibodies to $\beta 2$ GPI or human prothrombin. With the realization that APA, in fact, recognize proteins reconfigured on PL surfaces, a more accurate name for this family of antibodies is antiphospholipidprotein antibodies (APA).

APA may be found in a variety of different clinical settings. In patients with underlying autoimmune disease (e.g., systemic lupus erythematosus, rheumatoid arthritis, etc.) APA are often detected. The presence of these antibodies in autoimmune disease has been linked to a variety of clinical complications including recurrent venous and arterial thromboembolic events, recurrent abortion/fetal loss, and thrombocytopenia. The antiphospholipid syndrome (APS) is defined by

the presence of one or more of the clinical findings noted above together with positive laboratory tests for ACA or LA. However, the criteria required to diagnose APS need to be revised. With the realization that APA target PL-binding proteins, new test systems have been introduced including those which identify antibodies to specific proteins (e.g., $\beta 2$ GPI). Recently, patients with APS have been described who have negative test results for ACA and LA and only demonstrate antibodies to $\beta 2$ GPI⁷.

In addition to autoimmune APA, alloimmune APA have been described following various infections including bacterial, viral, and protozoal agents. Often, alloimmune APA are transient in contrast to persistence typically seen with autoimmune APA. **Table 2** summarizes clinical setting of allo- and autoimmune APA. An important characteristic of alloimmune APA is its transient nature. This is in contrast to persistence of APA in autoimmune disease (i.e., persistent

positivity of laboratory results demonstrated 6-8 weeks after the initial positive test).

Laboratory Detection of APA

Lupus Anticoagulants

The term lupus anticoagulants was first proposed by Feinstein and Rapaport in 1972⁸⁾. This term is a misnomer since the vast majority of patients with LA do not have underlying systemic lupus erythematosus (SLE). Many patients with LA are identified immediately following an intercurrent upper respiratory tract infection. These patients do not manifest any of the findings typically seen with APS. Subsequent testing will, in most cases, show disappearance of LA. In many cases, patients positive for LA will also have ACA positivity (~40%). Conversely, ACA positive patients, in many cases, do not have an associated LA. These differences may reflect the underlying quantity of antibody present in the patient plasma as well as differences in protein targets and antibody affinity.

Demonstration of LA identifies a patient at increased risk for thromboembolic events as well as recurrent spontaneous abortions (RSA)¹⁾²⁾⁹⁾¹⁰⁾¹¹⁾. The presence of LA in the setting of autoimmune disease indicates a relative risk for thrombosis of about seven-to eight-fold increase when compared to controls¹⁾¹²⁾. Approximately two-thirds of thromboembolic events are venous and the remaining one-third arterial. Arterial events are typically found in the cerebral circulation¹³⁾.

The laboratory diagnosis of LA requires a careful sequential series of steps¹⁴⁾. When a clinician requests the laboratory to rule out LA, it is very important for the laboratory to

also do parallel testing for ACA. The presence of persistently positive LA and/or ACA identifies patients at increased risk for thromboembolic events. Patients at risk may be positive for one or both of these tests (more recently, it has been appreciated patients may be negative for both tests and yet still have an increased risk of thrombosis due to anti- β 2 GPI). Thus, when clinicians request a LA evaluation, they are asking for a comprehensive evaluation for the presence of APA. The laboratory is responsible for performing the appropriate range of tests necessary to identify members of this family of antibodies¹⁾²⁾.

The Lupus Anticoagulant/Phospholipid-dependent Antibody Subcommittee of the Scientific and Standardization Committee/International Society of Thrombosis and Haemostasis (ISTH) has published specific guidelines required to identify LA. Four sequential steps are necessary¹⁵⁾.

(1) Demonstration of an abnormal phospholipid-dependent coagulation screening test (e.g., Kaolin Clotting Time [KCT], dilute Russell Viper Venom Time [dRVVT], Activated Partial Thromboplastin Time [APTT], Textarin Time [TT]) ;

(2) Demonstration the abnormal screening test is due to the presence of an inhibitor (synonym ; circulating anticoagulant) ;

(3) Proof the inhibitor is phospholipid-dependent ;

(4) Rule out other coagulopathies (e.g., presence of heparin in the sample, circulating inhibitors such as factor VIII inhibitor).

With increasing recognition of the complexity of the diagnosis of LA, a variety of new test systems have been introduced. In some cases, these tests are complex involving

incorporation of Steps 1 through 3 into a single system. The Staclot LA® is an example of such an integrated approach¹⁶⁾. Also, dRVVT-based systems have been marketed¹⁷⁾.

Because of the heterogeneity of the antibody population seen in patients with LA, more than one screening procedure is required. Most often, laboratories choose screening procedures which differ in the portion of the coagulation test system analyzed (e.g., APTT : Intrinsic ; PT : Extrinsic, dRVVT, Final Common Pathway). For instance, the most common combination is a dRVVT and APTT-based systems. Also, the dilute Prothrombin Time (dPT) and Textarin Time have been used¹⁸⁾.

Anticardiolipin Antibodies (ACA)

ACA testing was originally described utilizing solid phase radioimmunoassay¹⁹⁾. Because this assay system was expensive and also required utilizing radioisotopes, many laboratories quickly moved to ELISA technology²⁰⁾. As discussed earlier, ACA measures antibodies to $\beta 2$ GPI⁵⁾²¹⁾²²⁾. Other plasma proteins which bind to PLs may be identified in the conventional ACA assay (antibodies to Annexin V). $\beta 2$ GPI is a member of the short consensus repeat family of proteins. Physiologically it has anticoagulant properties (inhibits contact activation of the intrinsic pathway, inhibits ADP-induced platelet aggregation, inhibits the prothrombinase reaction). Despite its anticoagulant activities, hereditary deficiency of $\beta 2$ Glycoprotein I is not associated with a predisposition to thrombosis. Presumably, the mechanism of thrombosis associated with antibodies to $\beta 2$ Glycoprotein I requires transmembrane signaling with cellular activation

eliciting procoagulant reactions²³⁾. As noted above, antibodies to $\beta 2$ GPI are now being evaluated as a means of identifying patients at increased risk for thrombosis. Anti- $\beta 2$ GPI appears to be more specific in identifying patients at risk of thrombosis²⁴⁾. Consequently, many laboratories now perform both conventional ACA and an ELISA assay for antibodies to $\beta 2$ GPI.

Antibody-Mediated Thrombosis

APA are perhaps the most common acquired cause of thrombophilia. With the recent discovery of hereditary abnormalities leading to thrombophilia (e.g., factor V Leiden, 3' untranslated portion of the prothrombin gene [G20210A], etc.), laboratories are increasingly testing patients for both hereditary and acquired risk factors for thrombosis²⁵⁾. The concept of antibody-mediated thrombosis has been debated for a number of years. Perhaps the classic example is heparin-induced thrombocytopenia (HIT)²⁶⁾. HIT is associated with antibodies which appear to recognize a complex of heparin-platelet factor 4 (or other plasma proteins such as IL-6)²⁷⁾. This immune complex binds to Fc γ IIa receptors on the surface of platelets resulting in activation of platelets and subsequent thrombosis²⁸⁾. The pathophysiology of thrombosis seen in the APS is similar. In this case, however, $\beta 2$ GPI binds to activated cell membranes (with exposed phosphatidylserine). Antibodies then react with neoepitopes on bound $\beta 2$ GPI. This antigen antibody complex, in turn, activates Fc γ IIa receptors leading to platelet activation and thrombotic events (**Table 3**).

Also, APA may inhibit regulatory systems

Table 3 Similarities of APS & HIT*

	APS	HIT
Immunoglobulin	IgG ₂ *	IgG ₂ *
Epitope (Complexes)	PL-II PL- β 2 GPI	PF4-H
Receptor	Fc γ RIIa	Fc γ RIIa
Cellular Activation	Yes	Yes
Clinical	VT & AT	VT & AT

Legend: APS= Antiphospholipid Syndrome
HIT= Heparin Induced Thrombocytopenia
PL-II: Phospholipid-Prothrombin Complex
PF 4-H: Platelet Factor 4-Heparin Complex
VT: Venous Thrombosis
AT: Arterial Thrombosis

*Predominant immunoglobulin, often patients will have a mixture of immunoglobulin

based on membrane localized reactions. The protein C/protein S system requires activation of protein C and subsequent downregulation of factor Va and factor VIIIa by a complex of Activated Protein C (APC) and protein S on cellular membranes²⁹⁾⁽³⁰⁾. Antibodies to β 2 GPI can inhibit this reaction. Again, predisposing to thrombosis.

HIT and the antiphospholipid syndrome represents paradigms of antibody-mediated thrombosis³¹⁾. Other examples include the pathogenicity of antibodies to oxidized LDL and their association with accelerated atherosclerosis³²⁾. (**Fig. 1**) In addition, antibodies to components of the protein C system have been described (anti-PC and anti-PS as well as antithrombomodulin). Antiendothelial cell membrane antibodies have also been linked to thrombosis. In the coming years, additional mechanisms for antibody-mediated thrombosis will be described. It now appears antibody-mediated thrombosis may be one of the most important acquired mechanisms for thrombosis affecting the arterial and venous circulation.

Fig. 1 Antibody Induced Thrombosis
Legend: AECA = Antiendothelial Cell Antibodies
Anti-Ox-LDL = Antibodies to Oxidized Low Density Lipoproteins
Anti- β ₂ GPI = Antibodies to Beta₂ Glycoprotein I
Anti-Prothrombin = Antibodies to Prothrombin
APA = Antiphospholipid-Protein Antibodies

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