Clin Appl Thrombosis/Hemostasis 12(1):41–45, 2006

Antiphospholipid-Mediated Thrombosis: Interplay Between Anticardiolipin Antibodies and Vascular Cells

Chary López-Pedrera, PhD,* Paula Buendía, MSc,* Nuria Barbarroja, MSc,* Emilio Siendones, PhD,* Francisco Velasco, PhD,* and Maria José Cuadrado, PhD†

**Research Unit and Hematology Department, Reina Sofía University Hospital, Cordoba, Spain;*
†Lupus Research Unit, St Thomas' Hospital, London, United Kingdom

Summary: The antiphospholipid syndrome (APS) is characterized by thrombosis or pregnancy morbidity in the presence of antiphospholipid autoantibodies (aPL). aPL are a heterogeneous family of autoantibodies with diverse cross-reactivities whose origin and role have not been fully elucidated. Many of the autoantibodies associated with APS are directed against phospholipid-binding plasma proteins, such as β2-GPI and prothrombin, or phospholipid-protein complexes. The mechanisms by which aPL cause thrombosis are not completely understood. There is no unique mechanism able to explain all symptoms associated with the presence of aPL. Different theories have been proposed, including the effect of aPL on endothelial cells, monocytes, and platelets. aPL are able to recognize, injure, or activate cultured vascular endothelial cells. Cultured endothelial cells incubated with aPL express increased levels of cell adhesion molecules and tissue factor (TF), an effect mediated by β2-GPI, and may promote inflammation and thrombosis. Overexpression of TF has been also shown in monocytes in vitro and ex vivo. TF is the major initiator of coagulation in vivo; thus, its dysregulation may be one of the most important contributors to thrombosis. Effects of aPL upon platelets are not completely elucidated. aPL bind anionic phospholipid but they are normally in the inner side of cell membranes. When platelets are activated by different agonists, anionic phospholipids are exposed. There is some evidence showing that activated platelets are present in aPL-positive patients. Increased levels of β-thromboomodulin, and microvesicle formation seem to support this hypothesis. Activated platelets may contribute to thrombosis by persistent exposure of a procoagulant surface.
Key Words: Antiphospholipid syndrome—Antiphospholipid antibodies—Thrombosis—Vascular cells.

The antiphospholipid syndrome (APS) is an acquired autoimmune disorder of unknown etiology. The syndrome is defined by the association of arterial or venous thrombosis and/or pregnancy morbidity, in the presence of antiphospholipid antibodies (aPL): anticardiolipin antibodies (aCL) and lupus anticoagulant (LA) (1).

Ch.L.P was supported by a Science and Technology postdoctoral fellowship from the government of Spain. This work was supported by grants from the, the "Junta de Andalucía" (exp 171/03) and the "Fondo de Investigación Sanitaria" (FIS 03/1033) of Spain.

Address correspondence and reprint requests to Chary López-Pedrera, MSc, PhD, Unidad de Investigación, Hospital Universitario Reina Sofía, Avda. Menéndez Pidal s/n, E-14004 Córdoba, Spain; e-mail: rosario.lopez.exts@juntadeandalucia.es.

aPLs are a heterogeneous family of autoantibodies with diverse cross-reactivities whose origin and role have not been fully elucidated. Currently, the clinically important aPLs remain aCL and LA. aCL are defined by reactivity to cardiolipin bound to a solid phase, usually in an enzyme immunoassay. Internationally accepted criteria for the identification of LA require the following: (1) prolongation of at least one phospholipid-dependent coagulation assay (e.g., dilute Russell viper venom test), (2) failure to correct this inhibition of in vitro coagulation by the addition of normal plasma, and (3) correction of inhibition of in-vitro coagulation by the addition of phospholipid (2).

It is now well known that most aPL do not bind phospholipids but proteins complex to anionic phospholipids (3). In patients with APS,

most aCL detected with ELISA are directed against β2-glycoprotein I (β2-BPI), a 50-kDa plasma glycoprotein with anionic phospholipids-binding properties. Antibodies directed against prothrombin and also β2-GPI account for the majority of LA activity. A variety of other antibodies against different phospholipid-binding proteins have been recently named (Table 1) but their pathogenic mechanisms and clinical meanings are still uncertain (4).

There are some data coming from animal models, which support the pathogenic role of aPL. Mice immunized against β2-GPI (5) and with aPL antibodies (6) developed fetal loss. Also, mice infused with aPL developed significantly larger thrombi in femoral veins after experimental injury than mice infused with control antibodies (7).

Different mechanisms by which aPLs can lead to thrombosis have been described. Some of them were investigated before the recent clarification of autoantibody specificities and this can explain the different results obtained. Moreover, it seems clear that thrombosis in APS is a "two-hit" phenomenon. Antibodies—always present in blood—would cause a prothrombotic state but a second factor should be required to trigger thrombosis. Although speculative, this may include traumatic injury to the vascular bed, non-immunologic procoagulant factors, or the presence of infection leading to cytokine production and endothelial cell activation (8).

The diversity of antibodies and its specificities may also explain the wide spectrum of symptoms related to the presence of aPL and why some patients have only arterial thrombosis, others only venous thrombosis, or why only 30% of patients with positive aPL develop APS-related features.

The most likely mechanisms involving autoantibodies against phospholipid-binding proteins and leading to thrombosis can be classified as those by which aPLs interfere with hemostatic reactions (physiologic anticoagulant reactions and fibrinolysis) and those where antibodies induce cell-mediated events. The main cells involved are endothelial cells, monocytes, and platelets (4). This brief review is focused on mechanisms involving cell-mediated events.

TABLE 1. Phospholipid-binding Proteins

- β-glycoprotein I
- Prothrombin
- Protein C
- Protein S
- Annexin V
- Thrombomodulin
- Kininogen

ANTIPHOSPHOLIPID ANTIBODIES AND THE ENDOTHELIAL CELLS

Endothelial cell (EC) activation includes the change of its phenotype from antithrombotic to thrombotic, together with the production of adhesion molecules and cytokines. Several studies have investigated EC binding and activating properties of aPL antibodies. These studies showed binding of aPL to EC that might be mediated by β2-GPI (9,10). Anti–β2-GPI antibodies may bind to endothelial cells via β2-GPI bound to anionic phospholipids, such as phosphatidylserine in the cell membrane or to β2-GPI bound to β-receptor. Autoantibodies would transduce a signal by cross-linking of β-receptor or through binding to FcR, leading to the expression of adhesion molecules such as E-selectin, VCAM-1, and ICAM-1.

Increased expression of tissue factor (TF) in cultured vascular ECs by aPL has been described, with an increase in procoagulant activity (11). TF is a specific transmembrane single-chain glycoprotein composed of 263 amino acids (47 kDa), that requires interaction with specific membrane phospholipids to become functionally active (12–14). TF is widely accepted to be the major initiator of in vivo coagulation (15). It is also believed that TF has a key role in fibrin deposition in immunologic disorders and can be induced, in vitro, to appear on endothelial cells and monocytes in a transcriptionally regulated manner by several physiologic or non-physiologic stimuli (16–18). The same study describes an increase in monocyte adherence to EC, which also contributes to a hypercoagulable state and expression of adhesion molecules enhanced in EC. The interaction between endothelial and monocytic cells plays an important role in several thrombosis phases. A very recent study showed that monoclonal aCL and purified IgG fractions from patients with APS induce the production of monocyte chemoattractant protein-1 in human umbilical vein endothelial cells (HUVEC) (19). The authors conclude that aCLs promote endothelial cell–monocyte cross-talk by enhancing the endothelial production of monocyte chemo-

attractant protein-1 and therefore, switching the hemostatic balance to the prothrombotic state of APS.

ANTIPHOSPHOLIPID ANTIBODIES AND MONOCYTES

A number of recent studies link aPL to increased procoagulant activity on circulating blood monocytes, inducing the expression of procoagulant activity of TF. In vitro, plasma from patients with APS can induce procoagulant activity in HUVEC (20) as well as increased TF expression in normal monocytes (21). These effects seem to be restricted mostly to the plasma from patients with aPL and a history of thrombosis, whereas plasma from patients with aPL but no previous thrombosis could have no apparent effect (21). A positive correlation was observed between the ability of APS plasma to promote TF-dependent procoagulant activity and monocyte TF expression and levels of plasma thrombin-antithrombin III and prothrombin fragment 1 + 2, suggesting increased turnover of coagulation (21).

In vivo, upregulation of TF pathway in patients with APS has also been described (22–24). Our group has recently shown (23–25) that TF-related procoagulant activity and TF mRNA levels in mononuclear blood cells, as well as the cell-surface expression of TF on monocytes, are increased in primary APS patients with thrombosis when compared with those without thrombosis and with healthy controls. TF expression in these patients has been found to be further increased in those positives for IgG aCL (23).

The mechanisms by which aPL induce TF expression is unknown. The aCL responsible for the in vitro stimulation of TF exhibited anti–β2-GPI activity, and β2-GPI was present in the aCL-containing incubation media (22). However, β2-GPI binding to membranes containing physiologic concentrations of anionic aPL appears to be relatively weak. Roubey has suggested that anti–β2-GPI antibodies could stabilize the β2-GPI-anionic interaction by cross-linking membrane-bound β2-GPI (4). Also, binding of aPL to PL-bound proteins on the cell surface, apart from mediating cell activation via the FcγRII, may be in itself a synergistic (or alternative) way by which aPL induces TF expression.

More recently, Annexin II, a phospholipid-binding protein, has been identified as an EC or monocyte surface molecule and might be involved in aPL-mediated cellular signaling, playing a critical role in the up-regulation of monocyte TF (26).

At a molecular level, it has been suggested that aPL may interact with specific cell surface receptors (proteins and/or lipids), inducing signals that have consequences downstream, and that ultimately will result in up-regulation of cell surface proteins (i.e., TF). In an in vitro recent study, Pierangeli and associates demonstrated that aPL induces activation of the nuclear factor kappa by EC (27). In turn, NFKB activation leads to upregulation of gene transcription of adhesion molecules on EC and to initiation of various signal transduction pathways.

ANTIPHOSPHOLIPID ANTIBODIES AND PLATELETS

How aPL interacts with platelets contributing to a hypercoagulable state is not fully understood. It seems that a previous stimulus that activates platelets is an essential step for aPL binding to the platelet surface. Normal platelet membrane shows a clear phospholipid asymmetry with phosphatidylserine predominantly located in the cytoplasmic leaftlet of the membrane. When platelets are activated, lose their physiologic phospholipid asymmetry, and increase the exposure of anionic phospholipids, mainly phosphotidylserine on the external membrane. Thus, circulating aCL/β2-GPI complexes might bind directly to phosphatidylserine on the activated platelet membrane (28).

Numerous investigators have previously studied platelets' status in patients with aPL and some suggested that these antibodies may enhance thrombin-induced platelet activation/aggregation (29).

Increased excretion of platelet-derived thromboxane urinary metabolites has been reported in aPL-positive patients who had antibodies to β2-GPI (30). Also, the platelet activation markers CD62p and CD63 have been studied in aPL positive patients (31). CD63 was significantly increased in patients with primary APS compared with healthy donors. There was no significant difference in median CD62p expression or percentage reticulated platelets between the two groups. Interestingly, CD63 values were lower in patients receiving aspirin, but aspirin did not prevent significant platelet activation occurring in some patients. Also, the median levels of P-selectin were significantly higher in patients than

in controls. The lack of difference in platelet membrane CD62p expression may be explained because platelets expressing higher levels of CD62p might bind to monocytes and/or neutrophils and so excluded flow cytometry analysis. Platelet activation also leads to alterations in the GPIIb/IIIa receptor complex, formation of platelet-leukocyte aggregates and platelet-derived microparticles, although the molecular and cellular mechanism leading to the activation and regulation of these events remains unknown.

CONCLUSION

Thrombosis or pregnancy morbidiy and positivity of antiphospholipid antibodies define the features of antiphospholipid syndrome. Potential mechanisms of thrombosis in APS include aPL engagement of antigens on cell surfaces, leading to signal transduction and altered cell activity. This cell activation manifests in several ways, including enhanced adhesion molecule expression on endothelial cells, increased leukocyte adherence to ECs, increased procoagulant activity on ECs and monocytes, and enhanced thrombin-induced platelet activation/aggregation after aPL activation. Ongoing studies will show how each of these mechanisms contribute to the hypercoagulable state of patients with APS.

REFERENCES

1. Wilson WA, Gharavi AE, Koike T, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: Report and international workshop. *Arthritis Rheum* 1999;42:1309.
2. Brandt JT, Douglas A, Triplett AD, et al. Criteria for the diagnosis of lupus anticoagulants: An update. *Thromb Haemost* 1995;74:1185.
3. Galli M, Comfurius P, Maassen C, et al. Anticardiolipin antibodies (ACA) are directed not to cardiolipin but to a plasma protein cofactor. *Lancet* 1990;335:1544.
4. Roubey RAS. Immunology of the antiphospholipid antibody syndrome. *Arthritis Rheum* 1996;39:1444.
5. Garcia CO, Kanbour-Shakir A, Tang H, et al. Induction of experimental antiphospholipid antibody síndrome in PL/J mice following immunization with β-GPI. *Am J Reprod Immunol* 1997;37:118.
6. Blank M, Faden D, Tincani A, et al. Immunization with anticardiolipin cofactor (β-glycoprotein I) induces experimental antiphospholipid syndrome in naïve mice. *J Autoimm* 1994;7:441.
7. Pierangeli SS, Harris EN. In vivo models of thrombosis for the antiphospholipid syndrome. *Lupus* 1996;5:451.
8. Hanly JG. Antiphospholipid syndrome: An overview. *CMAJ* 2003;168:1675.
9. Del Papa N, Guidali L, Spatola L, et al. Relationship between anti-phospholipid and anti-endothelial cell antibodies: β-glycoprotein I mediates the antibody binding to endothelial membranes and induces the expression of adhesion molecules. *Clin Exp Rheumatol* 1995;13:179.
10. Del Papa N, Guidali L, Sala A, et al. Endothelial cells as target for anti-phospholipid antibodies. *Arthr Rheum* 1997;40:551.
11. Simantov R, LaSala JM, Lo SK, et al. Activation of cultured vascular endotelial cells by antiphospholipid antibodies. *J Clin Invest* 1995;96:2211.
12. Nemerson Y, Gentry R. An ordered addition, essential activation model of the tissue factor pathway of coagulation: Evidence for a conformational cage. *Biochemistry* 1986;25:4020.
13. Nemerson Y. Tissue factor and hemostasis. *Blood* 1988;71:1.
14. Rodgers GM. Hemostatic properties of normal and perturbed vascular cells. *FASEB J* 1988;2:116.
15. Weis JR, Pitas RE, Wilson BD, Rodgers GM. Oxidized low-density lipoprotein increases cultured human endothelial cell tissue factor activity and reduces protein C activation. *FASEB J* 1991;5:2459.
16. Gregory SA, Morrissey JH, Edgington TS. Regulation of tissue factor gene expression in the monocyte procoagulant response to endotoxin. *Mol Cell Biol* 1989;9:2752.
17. Mackman N. Regulation of the tissue factor gene. *FASEB J* 1995;9:883.
18. Courtney MA, Haidaris PJ, Marder VJ, Sporn LA. Tissue factor mRNA expression in the endothelium of an intact umbilical vein. *Blood* 1996;87:174.
19. Cho CS, Cho ML, Chen PP, et al. Antiphospholipid antibodies induce monocyte chemoattractact protein-1 in endothelial cells. *J Immunol* 2002;168:4209.
20. Branch DW, Rogers GM. Induction of endothelial cell tissue factor activity by sera from patients with antiphospholipid syndrome: A possible mechanism of thrombosis. *Am J Obstet Gynecol* 1993;168:206.
21. Reverter JC, Tassies D, Font J, et al. Hypercoagulable state in patients with antiphospholipid syndrome is related to high induced tissue factor expression on monocytes and to low free protein S. *Arterioscler Thromb Vasc Biol* 1996;16:1319.
22. Amengual O, Atsumi T, Khamashta MA, et al. The role of tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid síndrome. *Thromb Haemost* 1998;79:276.
23. Cuadrado MJ, Lopez-Pedrera CH, Khamashta MA, et al. Trombosis in primary antiphospholipid síndrome: A pivotal role for monocyte tissue factor expression. *Arthr Rheum* 1997;40:834.

24. Dobado-Berrios PM, Lopez-Pedrera CH, Velasco F, et al. Increased levels of tissue factor mRNA in mononuclear blood cells of patients with primary antiphospholipid síndrome. *Thromb Haemost* 1999;82:1578.

25. Dobado-Berrios PM, Lopez-Pedrera CH, Velasco F, et al. The role of tissue factor in the antiphospholipid syndrome. *Arthr Rheum* 2001;44:2467.

26. Zhou H, Roubey RAS. Annexin A2 and antiphospholipid-antibody induced upregulation of monocyte tissue factor. *Arthr Rheum* 2002;46:232

27. Pierangeli SS, Harris EN. Probing antiphospholipid-mediated thrombosis: The interplay between anticardiolipin antibodies and endothelial cells. *Lupus* 2003;12:539.

28. Galli M, Bevers EM, Comfurius P, et al. Effect of antiphospholipid antibodies on procoagulant activity of activated platelets and platelet-derived microvesicles. *Br J Haematol* 1993;83:466.

29. Cambell AI, Pierangeli SS, Wellhausen S, et al. Comparison of the effects of anticardiolipin antibodies from patients with the antiphospholipid syndrome and with syphilis on platelet activation and aggregation. *Thromb Haemost* 1995;73:529.

30. Forastiero R, Martinuzzo M, Carreras L, et al. Anti-β-glycoprotein I antibodies and platelet activation in patients with antiphospholipid antibodies: Association with increased excretion of platelet-derived thromboxane urinary metabolitos. *Thromb Haemost* 1998;79:42.

31. Joseph JE, Harrison P, Mackie IJ, et al. Platelet activation and turnover in the primary antiphospholipid syndrome. *Lupus* 1998;7:333.