Antiphospholipid Antibodies and Thrombotic Predisposition: Underlying Pathogenetic Mechanisms

By Samuel A. Santoro

LUPUS ANTICOAGULANTS (LA), anticardiolipin (aCL) antibodies, the antiphospholipid (aPL) syndrome, and the associated predisposition to thrombosis pose many difficulties ranging from practical issues of detection and patient management to conceptual issues regarding the mechanism(s) underlying the associated thrombotic tendency. aPL antibodies represent a family of IgGs with reactivity for anionic phospholipids. Most attention has been centered on LA, which are detected by virtue of their ability to inhibit phospholipid-dependent coagulation assays and aCL, which are detected by enzyme-linked immunosorbent assay (ELISA). Several recent studies have established the role of \( \beta_2 \) glycoprotein I as a necessary cofactor for detection of aCL antibodies.

Triplet has recently summarized issues relating to the laboratory analysis of LA and aCL. It is clear that laboratory tests for LA and aCL do not necessarily detect the same populations of antibodies. Some patients with aCL antibodies do not exhibit LA activity and vice versa. Of patients positive for LA or aCL, only about 60% are positive for both. As differing sensitivities of aCL and LA assays do not appear to account for the discordance, it seems most likely that aCL and LA represent two overlapping populations of antibodies. Whereas considerable progress has been made toward standardizing aCL assays, testing for LA remains more problematic.

A consensus panel has recently developed minimal guidelines for LA testing which include establishing that an abnormality of phospholipid-dependent coagulation reactions is present, that the abnormality is caused by an inhibitor rather than by factor deficiency, and that the inhibitor is directed at phospholipid rather than specific coagulation factors.

Despite the problems with laboratory testing, a large and still growing body of data has accumulated to establish associations between the presence of LA and/or aCL antibodies with thrombosis. Venous and arterial thrombosis, thrombocytopenia, and fetal wastage have all been associated with the presence of aPL antibodies. A key question that remains unanswered is whether the aPL antibodies are causal, coincidentally associated epiphenomena, or the consequence of as-yet-unspecified tissue damage. Two approaches appear especially useful to address this issue. One relies on careful, long-term, prospective analysis of relevant populations to establish whether the appearance of aPL antedates, appears concurrently with, or follows thrombotic episodes. Because the studies that established the association of aPL and thrombosis have been almost exclusively of retrospective design, they do not permit this question to be answered.

A second approach, only beginning to be exploited, is the experimental. One recent, provocative use of this approach is the study of Sthoege et al who showed that passive immunization of mice with a murine aCL monoclonal antibody reproduced the fetal wastage associated with aCL antibodies in humans. Similar controlled, experimental approaches to the study of other phenomena associated with aPL antibodies should play an important role establishing the pathogenetic role of the antibodies.

If (and that could still be a rather large if) the aCL and LA antibodies cause the thrombotic episodes associated with the aPL syndrome, what are the relevant pathogenetic mechanisms? Several hypotheses have been put forward, most focusing on the antiphospholipid specificity of the antibodies. These include interference with the production and release of prostacyclin by endothelial cells, interference with the regulatory protein C and protein S pathways, inhibition of the action of phospholipid placental anticoagulant protein-I (PAP-1), endothelial cell damage and activation, activation of platelets by the antibodies, impairment of fibrinolytic mechanisms, and a report of interference with the activity of antithrombin III. None of these proposed mechanisms has gained widespread acceptance as the underlying mechanism. For several, conflicting and contradictory evidence has been presented. Although space does not permit a detailed analysis of the proposed mechanisms, it seems fair to conclude that a satisfactory mechanistic explanation at the molecular level for the association between aCL, LA, and thrombosis has thus far eluded us.

The recent report of Ginsberg et al might provide an important clue. In their cross-sectional analysis of 43 consec-

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utive patients with systemic lupus erythematosus, they observed that patients with persistently positive aCL antibodies exhibited elevated levels of prothrombin fragment F(1 + 2) and fibrinopeptide A (FPA). As the F(1 + 2) fragment is cleaved from prothrombin by factor Xa of the prothrombinase complex, the level of F(1 + 2) reflects thrombin formation. Because FPA is cleaved from fibrinogen by thrombin, FPA can serve as an index of in vivo thrombin activity. FPA levels may increase because of increased thrombin generation and/or impaired inhibition of thrombin. These markers have been used in studies of antithrombin III, protein C, and protein S deficiency states to develop the concept that patients with familial conditions that predispose to thrombosis generate higher baseline levels of thrombin, even in the absence of clinically apparent thrombosis. By this standard, the data of Ginsberg et al. suggest that patients with aCL antibodies in a prothrombotic state.

How might enhanced thrombin levels arise in the presence of aPL antibodies? In this issue, the report of Shibata et al. may provide, at least in part, an explanation. Shibata et al. have observed that IgG with aCL activity purified from patients with aPL syndrome also bound heparin. IgG from controls did not exhibit readily detectable antiheparin activity. Shibata et al. went on to make the provocative observation that the antibodies were specifically reactive with a disaccharide sequence present with the region of heparin/ heparan sulfate recognized by antithrombin III. Furthermore, they demonstrated that the aCL/antiheparin antibodies inhibited in vitro the heparin accelerated formation of antithrombin III/thrombin complexes. It seems likely that only a subset of aCL antibodies also bind heparin because affinity chromatography of the IgG fraction on heparin-Sepharose (Pharmacia, Piscataway, NJ) produced a greater enrichment in antiheparin reactivity than aCL reactivity.

Mechanistically, the observations of Shibata et al. could account, at least in part, for the elevated thrombin activity present in the aPL syndrome. Given the multiple actions of thrombin in promoting coagulation, conversion of fibrinogen to fibrin, activation of factors V, VIII, and XI, and activation of platelets, the antiheparin/heparan sulfate activity of aCL antibodies could contribute to both the elevated F(1 + 2) and FPA levels observed by Ginsberg et al. Shibata et al. conclude that "considering the known physiologic importance of heparin sulfate/heparin in normal anticoagulation, antiphospholipid antibodies with high affinity for heparin, or antiheparin antibodies, may be an important cause of autoimmune vascular thrombosis in the antiphospholipid antibody syndrome."

Although it is early in the course of this work and the initial study is small, Shibata et al. may well have made an important advance in understanding the pathogenetic mechanisms underlying the predilection to thrombosis associated with aPL antibodies. Like many good, early investigative efforts, the study raises at least as many questions as it answers. For example, is thrombotic risk selectively increased in patients displaying the subset of aCL antibodies that react with heparin? If so, the finding may have important diagnostic and treatment implications. Might the presence of such antibodies complicate therapy with heparin? Is there a way around the need to first purify IgG before assaying for antiheparin activity? Such improved methodology will almost certainly be required to undertake the large population studies (both cross-sectional and prospective) needed to extend the initial observations of Shibata et al. Can well-characterized monoclonal antibodies displaying aCL and antiheparin activity be developed and used in an animal model to establish the hypothesis that the antibodies are a proximate cause of thrombosis?

The work of Shibata et al. described in this issue may provide new insight into the pathology of thrombosis in the aPL syndrome and direct focus away from the aPL reactivity per se of the antibodies. However, only after the expenditure of much more effort and the passage of time will we know if the report of Shibata et al. really is a step in the right direction.

REFERENCES
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