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Are post-translational modifications of β_2 -glycoprotein I markers for thrombotic risk? Are they triggers of autoimmunity?

Michael D. Lockshin and Jane E. Salmon

The Barbara Volcker Center and the Mary Kirkland Center for Lupus Research, The Hospital for Special Surgery and the Weill-Cornell Medical Center, 535 E 70th Street, New York, New York 10021, Tel 212-606-1461, Fax 212-774-2374

Michael D. Lockshin: LockshinM@hss.edu

For over 30 years, we have known that thrombosis and fetal loss are the signature features of antiphospholipid syndrome (APS). We understand the major functional components of this syndrome: antibodies, primarily of IgG class, bind to an autoantigen, in many cases β_2 -glycoprotein I (β_2 -GPI), that itself binds to either negatively charged phospholipids or, more likely, to cell surface receptors such as ApoER2. These events initiate a chain of intracellular or extracellular signals that lead to thrombosis.^{1–5} Although the chemistry and cell biology is familiar, it has not been possible to predict in whom and at what point in time patients with antiphospholipid antibodies will have clinical events.

Multiple studies in animals and humans elucidate elements of the pathophysiology that lead to a diagnosis of clinical APS. The hypotheses tested by the models focus on specific events from the first appearance of antibody to thrombosis, including phenotypic changes in vulnerable tissue, antibody triggered pathways of inflammation and injury, alterations in coagulation proteins and platelets, cellular activation by stimulation of surface receptors, environmental insults, and coexistent genetic thrombotic states.

The most robust current theories of pathogenesis incorporate fundamental observations: some form of cell activation occurs, exposing negatively charged phospholipids on the cell surface or initiating cell adhesion or thrombosis; β_2 -GPI is involved in the thrombotic process, even though antibody to β_2 -GPI is not a strong marker of risk; and trigger factors provoke clinical events.^{6, 7} Early involvement of inflammation, particularly complement activation, are established in animal models but are less clear in humans.

In this issue of *Arthritis & Rheumatism*, Ioannou and colleagues add to a body of work that secures for β_2 -GPI a central role in the pathophysiology of APS and argues that alterations in β_2 -GPI are associated with increased risk for thrombosis.⁸ β_2 -GPI (also known as apolipoprotein H), an incompletely understood protein, its physiological function incompletely defined, circulates in large quantity in plasma. Patients deficient in β_2 -GPI appear to be normal, compounding the mystery of its role. A part of the complement control protein family, β_2 -GPI consists of five “sushi domains” (wrapped like pieces of sushi). The molecule is J-shaped. Its hook end, the fifth domain, is its attachment site to the cell surface. The highly positively charged fifth domain binds to negatively charged surfaces, such as the phosphatidylserine that translocates from the inner cell membrane to the surface in activated or apoptotic cells. More recently, studies indicate that the fifth domain of β_2 -GPI also binds to ApoER2 receptors and thereby activates platelets and endothelial cells. Upon binding, β_2 -GPI undergoes a conformational change that likely explains its transformation from a benign circulating protein to an active participant in thrombosis.⁴ While the fifth domain targets β_2 -GPI to cell surfaces, the first domain contains a neoepitope, exposed after the molecule binds to a surface, that is recognized by antiphospholipid antibodies. Binding and cross-

linking of the first domain by antibodies trigger intracellular and extracellular pathways that result in thrombosis and may cause pregnancy complications.

In the current study, Ioannou and colleagues ask whether post-translational modification of β_2 -GPI, independent of binding-induced conformational change, correlates with thrombosis. They pose the question through a clinical study, examining total β_2 -GPI and the proportion of oxidized β_2 -GPI, oxidation defined as lacking free thiols, in a large multicenter study. Patients with non-thrombotic autoimmune disease, thrombosis without autoimmune disease, and healthy normals served as controls. Pregnancy loss as a manifestation of APS was not considered, although its mechanism may (or may not) be different. Indeed, most women with APS-related fetal loss do not suffer from thromboses.

The authors found that APS patients have higher levels of total β_2 -GPI and proportionally more oxidized β_2 -GPI than any of the control groups. In addition, oxidized β_2 -GPI was associated with lupus anticoagulant, an important observation because lupus anticoagulant is the dominant predictor of thrombotic clinical events in APS. The authors propose that relative levels of oxidized β_2 -GPI can stratify thrombotic risk in patients. However, they were not able to develop a more complete risk profile because the study, which included 139 APS patients, did not address the effects of SLE, anticoagulation, clinical manifestations of APS, or risk associated with a decrease in reduced β_2 -GPI. The authors speculate that clinical states characterized by increased oxidative stress, such as pregnancy and infection, produce elevated levels of oxidized β_2 -GPI which is more immunogenic and likely to break tolerance, and that the relative decrease in reduced β_2 -GPI leads to increased thrombotic risk. Importantly, in vitro studies from this group provide evidence that reduced β_2 -GPI protects endothelial cells from oxidative injury and maintains their viability.⁹ These studies identify a new and pivotal role for β_2 GP-I in the induction of thrombosis in APS.

The work of Ioannou and his group demonstrates clear biochemical differences between normal and pathogenic β_2 -GPI, links these differences to clinical phenotype, and suggests post-translational mechanisms by which β_2 -GPI can be modified to become pathogenic. Elucidating the structural biology of β_2 -GPI is one piece of the puzzle that we now know has broad implications. To understand pathophysiology and use relative levels of the post-translationally modified forms of β_2 -GPI for risk stratification, one also must consider antiphospholipid antibody class, antigen localization, activation of effector cells, initiation of pathways inflammation (complement and cytokines) and coagulation, and environmental triggers.

A number of questions about antiphospholipid syndrome cannot be answered by the observations of Ioannou et al. Why do some patients with high titer antiphospholipid antibody have no illness? How does oxidation of β_2 -GPI relate to familial and ethnic skewing in APS? Is β_2 -GPI more antigenic in its oxidized state? Does oxidized β_2 -GPI explain the basis for association of antiphospholipid antibodies with systemic lupus erythematosus? Is there a more effective treatment that blocks upstream events that will supplant the anticoagulation?

Larger studies with longitudinal follow up must confirm and extend the exciting new findings of Ioannou and his group. The onus is on the international community of physicians and scientists who care for and study APS patients to answer these questions.

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Table 1**Theories of how antiphospholipid antibodies cause APS**

Aspects of antiphospholipid antibodies that may contribute to pathogenicity	
<ul style="list-style-type: none">• Features of the autoantibody: class, subclass, or function that predisposes to thrombosis• Features of the antigen recognized: type of phospholipid or coagulation factor• Exposure of neoepitope in β_2-GPI associated with conformational changes• Signaling via endothelial surface receptors, including ApoER2, Annexin 2, TLRs• Activation of endothelial cells or platelets, including expression of tissue factor, adhesion molecules and inhibition of eNOS• Initiation of inflammation by targeted antibody, including complement activation and release of pro-inflammatory cytokines• Increased vulnerability of endothelium triggered by oxidative stress, oral contraceptives, surgery, infections, smoking, etc.• Other pro-thrombotic abnormalities, including MTHFR, factor V Leiden, etc.	