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Meeting report

Summary of the 11th International Congress on Antiphospholipid Antibodies, Sydney, Australia, November 2004

The 11th International Congress on Antiphospholipid Antibodies was held on 14–18th November 2004 in Sydney, Australia. The local host was Steven Krilis, a physician–scientist whose work on the characterization and function of beta2-glycoprotein I is second to none. The Congress's International Advisory Committee consisted of investigators from every continent, and scientists and clinicians from around the globe were present. As expected, Sydney proved to be a beautiful metropolitan venue located on the spectacularly picturesque harbour. Dr. Krilis and his local co-workers did a wonderful job of organizing a four-day conference featuring a full menu of basic science and clinical presentations, including numerous original investigations and state-of-the-art reviews.

The first day focused on a review and open discussion of the Sapporo Criteria for the diagnosis of certain antiphospholipid syndrome (APS). These criteria were developed at the 9th International Meeting in Sapporo, Japan, in 1999, and are analogous to the American Rheumatism Association Criteria for Systemic Lupus Erythematosus. Thus, they form the generally accepted basis of the diagnosis (clinical and laboratory) of APS. Dr. Krilis had asked recognized authorities to review the scientific support for the current criteria and to provoke a healthy discussion regarding clinical and laboratory features potentially worthy of inclusion or revision. Numerous topics were covered, including cardiac, obstetric, neurologic, cutaneous, renal and hematologic clinical features, and current and new laboratory tests. Audience participation was robust throughout. A Workshop summary consensus statement updating the international classification criteria is being prepared. Highlights from this Workshop included:

- It was recommended that IgG and IgM anti-beta2-glycoprotein I antibodies be added to the current antiphospholipid antitodies (lupus anticoagulant and IgG and IgM anticardiolipin anibodies) for the diagnosis of APS.
- Several clinical associations not currently included as clinical criteria for the diagnosis of APS are widely recognized but could not be adopted due to lack of sufficient supporting data. Instead, the Workshop proposed carefully defined patient subsets of "an-

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tiphospholipid antibody-associated disease" for cardiac valve disease, livedo reticularis, nephropathy and thrombocytopenia.

- Several laboratory associations were treated in a similar fashion. These include IgA anti-beta2-glycoprotein I, IgA anticardiolipin, anti-phosphatidylserine, anti-phosphatidylethanolamine, antibodies against prothromin and antibodies to the phosphatidylserine–prothrombin complex. The Workshop encouraged interested investigators to design studies to clarify the role of these antibodies in APS and to intensify standardization efforts.
- Though fetal death and recurrent miscarriage were regarded as accepted clinical criteria, the sensitivity and specificity of preterm birth due to severe preeclampsia or placental insufficiency were questioned. The Workshop agreed that the association of antiphospholipid antibodies to preeclampsia, and perhaps placental insufficiency, is weak. Nonetheless, Workshop members felt that the current criterion should be left in place, the definition of placental insufficiency should be clarified and investigators should be encouraged to perform appropriate validation studies.

The first full day of the Congress included an engaging, state-of-the-art discussion of beta2-glycoprotein I. Most experts now believe that this highly conserved and abundant glycoprotein is the principle target of relevant antiphospholipid antibodies. It reacts with negatively charged surfaces, including anionic phospholipids, through its lysine-rich fifth domain (domain V). Though the exact role of this glycoprotein in coagulation is uncertain, the addition of beta2-glycoprotein I to plasma results in a prolongation of thrombin generation and a murine knockout model demonstrates impaired thrombin generation. Beta2-glycoprotein I-deficient humans are not at risk for thrombosis. It is now clear that beta2-glycoprotein I has both procoagulant and anticoagulant functions. It binds to anionic phospholipids surfaces, perhaps competing with coagulation factors. Cleavage in domain V at Lys317-Thr318 by plasmin or activated factor X abolishes binding to anionic phospholipids and decreases beta2-glycoproten I inhibition of factor XI activation. Cleavage also inhibits plasmin generation by tissue plasminogen activator, linking beta2-glycoprotein I to the fibrinolytic system.

Roles for beta2-glycoprotein I in APS are suggested by a number of recent observations. APS autoantibodies have relatively low affinity for domain 1 of the glycoprotein. However, clustering of beta2-glycoprotein on the surface of activated platelets or other cells may allow bivalent binding of the antiphospholipid antibodies to adjacent molecules and, in effect, create dimeric beta2-glycoprotein I. Experimental evidence suggests that dimeric antibody to beta2-glycoprotein I binds to platelet ApoER2, a cell surface receptor, and enhances platelet sensitivity to thrombin.

What role beta2-glycoprotein I might play in fetal loss remains uncertain. Beta2-glycoprotein I knockout mice have normal pregnancy outcomes. However, beta2-glycoprotein I-deficient mice have a reduction in litter size and subtle changes in the placenta that might represent impaired trophoblast development or function. Dr. Krilis believes currently that antiphospholipid antibodies may be pathogenic, at least with regard to pregnancy, by potentiating rather than abolishing some effects of beta2-glycoprotein I.

Investigators from Keio University in Tokyo presented original data regarding the presence of autoreactive, beta2-glycoprotein I-specific CD4+ T cells. These cells rec-

ognize peptide sequences of beta2-glycoprotein I in the context of HLA-DR53 and stimulate anti-beta2-glycoprotein I production by B cells. The cells respond to reduced beta2-glycoprotein I levels and to recombinant fragments of the molecule, but not to native beta-2-glycoprotein I. The investigators interpret this to mean that the autoreactive cells recognize "cryptic" determinants generated by processing of native beta2-glycoprotein I. Though present in normal individuals and in APS patients, the autoreactive CD4+ cells are activated in patients with APS, suggesting a possible pathogenic role.

Experts reviewed other subjects and enlightened the audience with new findings. Joyce Rauch presented evidence that human polyclonal and murine monoclonal lupus anticoagulants bind to prothrombin bound to apoptotic Jurkat cells and suggested that binding potentiates the procoagulant effect of apoptotic cells. Jacob Rand reviewed the role of annexin A5 as an "anticoagulant shield" on trophoblast. His experimental evidence suggests that antiphospholipid antibodies with high affinity for phospholipids may interfere with the assembly of annexin A5 on trophoblast. Robert Roubey discussed the role for autoantibody-induced monocyte and endothelial tissue factor expression as a cause of thrombosis in APS, providing proof of increased transcription and translation of nascent tissue factor as the mechanism of increased expression. Silvia Pierangeli discussed experiments performed by her and her colleagues showing that fluvastatin downregulates tissue factor expression induced by TNF-alpha in monocytes. Her group has shown that fluvastatin reduces thrombosis size and duration in their murine pinch-thrombosis model and also reduces leukocyte vascular wall adhesion. Fluvastatin additionally reduces tissue factor expression induced by antiphospholipid antibodies in human endothelial cell cultures. In collaboration with Drs. Salmon and Giardi from Cornell, Dr. Pierangeli has shown that complement activation plays an important role in thrombosis in a murine model in which thrombosis is initiated via a standardized "pinch" mechanism. Other basic science presentations on the first day revealed that platelet glycoprotein IIB/IIIA plays a role in antiphospholipid antibody-induced thrombosis and that beta2-glycoprotein I-null mice have a defect in placental architecture but no increase in fetal wastage or placental thrombosis. Dr. Shoenfeld's group supported an infectious etiology for APS by showing that some anti-beta2-glycoprotein I antibodies are directed to the glycosylated site of the beta2-glycoprotein molecule which cross-reacts with peptides derived from infectious yeast.

There were numerous presentations on clinically oriented data. Dr. Steve Levine, presenting for the WARSS/APASS group, noted that although the concordance of lupus anticoagulant or anticardiolipin antibodies with anti-beta2-glycoprotein I or anti-phosphatidylserine antibodies is low, being positive for either lupus anticoagulant or anticardiolipin increased the likelihood of being positive for anti-beta2-glycoprotein I or anti-phosphatidylserine antibodies. A possible increased risk for thrombo-occlusive events in individuals positive for anti-beta2-glycoprotein I or anti-phosphatidylserine suggests the need for additional studies of these antibodies. Dr. Tektonidou's presentation regarding nephropathy in patients with APS prompted considerable discussion and debate, particularly concerning the histologic features of this condition. Two studies from French investigators found that heat shock protein-60 reactivity is associated with antiphospholipid antibodies in vasculitis-related autoimmune disorders. Drs. Sulistyono and Dachlan from Indonesia reported that eclampsia is associated with anticardiolipin antibodies.

The second day of the meeting started off with a session on pregnancy. There were excellent reviews of the function of the human placenta, maternal hemostasis and potential immunologic manipulations of the fetus. I presented a review of the evidence supporting and questioning the diagnostic categories for APS-associated reproductive outcomes as well as the randomized trial evidence for treatment of APS during pregnancy with a variety of agents. This was followed by Drs. Lassere and Empson's systematic review of therapeutic trials. Their conclusion was that a cooperative and large, fully-blinded placebo controlled trial is still desperately needed. To my surprise, a good number of attendees appeared quite positive about this seemingly radical suggestion.

Very good presentations by young investigators followed. Investigators from Australia and the US showed that beta2-glycoprotein I binds the C-terminal domain of factor XI to inhibit its activation by thrombin and factor XIIa and that plasmin cleavage of the glycoprotein abolishes this activity. The group from University College in London showed that antiphospholipid antibodies reduce in vitro cleavage of domain V of beta2-glycoprotein I by plasmin. The group from the Netherlands presented studies as to how relevant antiphospholipid antibodies recognize epitopes on domain I of beta2-glycoprotein I as well as epitopes on domain V. Members of the same group also showed that dimerization of beta2-glycoprotein I results in increased adhesion of platelets to collagen and that this same dimeric molecule binds to ApoER2, a member of the LRP receptor family present on platelets. Investigators from Dr. Pierangeli's laboratory at Morehouse in Atlanta showed that p38 mitogen-activated protein kinase plays a role in the up-regulation of tissue factor by antiphospholipid antibodies. New Zealand investigators provided in vitro data showing that heparins induce a significant and concentration-dependent positive effect on antiphospholipid antibody-inhibition of trophoblast proliferation, suggesting a novel mechanism for the clinical benefit of heparin in APS.

On the clinical front, a Japanese study found that measuring anti-phosphatidylserine or anti-prothrombin antibodies was not associated with recurrent miscarriage. Marie-Claire Boffa reported on her registry of infants born of mothers with APS—a web-based registry that is in progress. Robert Roubey updated the audience on the US APS registry, with now over 650 well-characterized individuals enrolled. Relevant clinical details, including laboratory correlations, were reported. Michael Lockshin critically reviewed the controversy surrounding how much warfarin is enough for patients with APS-related thrombosis and Dr. Crowther discussed his study of the subject and outlined the next steps in these important clinical trials. Dr. Erkan updated the group on his APLASA study of thrombosis in previously asymptomatic individuals with antiphospholipid antibodies, indicating that the prospective risk of thrombosis appears low (2.5% or less) and, thus far, there is no difference between aspirin-treated and untreated patients.

The following morning covered aspects of pathophysiologic mechanism of antiphospholipid antibodies and CNS and cardiovascular manifestations. Ron Derksen eloquently discussed how dimeric beta2-glycoprotein I binds to the platelet LDL receptor, ApoER2, to cause induced p38MAPK phosphorylation, confirming that binding actually signals cells and activates platelets. The Dutch group has also shown that dimeric beta2-glycoprotein I binds to other LDL receptor family members, raising the possibility that this may be responsible for manifestations of disease other than thrombosis. Silvia Pierangeli and her group showed that antiphospholipid antibody-induced platelet

thromboxane production and endothelial cell activation are abrogated by a p38MAPK inhibitor, confirming this as an exciting area for future research. Drs. Yasuda, Atsumi and Koike showed that cleavage of beta2-glycoprotein I in domain V results in suppression of plasmin generation by binding to plasminogen, tying in the fibrinolytic pathway in APS.

Regarding the CNS manifestations of APS, Dr. Chapman of Sheba Medical Center in Israel pointed out controversies surrounding the possible relationship between APS and multiple sclerosis. Dr. Gatenby of Canberra, Australia, critically analyzed the link between antiphospholipid antibodies and stroke, concluding that there is much left to do in order to understand and optimally treat stroke in individuals with antiphospholipid antibodies. Robin Brey's insightful review of the management of CNS manifestations of APS noted that the role of antiphospholipid antibodies in recurrent stroke is uncertain and that moderate-intensity warfarin is as effective as high-intensity warfarin or aspirin for the prevention of recurrent stroke in APS. Both the San Antonio, Texas group and the group from McMaster University in Canada presented evidence that antiphospholipid antibodies are independently associated with SLE-related cognitive dysfunction over time.

Dr. Cervera reviewed the relationship between antiphospholipid antibodies and coronary and valvular syndromes. He concluded that current evidence does not allow us to determine the exact mechanism of cardiac lesions in APS patients and suggested that serial screening in longitudinal studies will be required. Dr. Font and other members of the Barcelona group studied the incidence and progression of severe valvular disease in patients with SLE and concluded that the presence of high levels of anticardiolipin antibodies are associated with development of severe valvular regurgitation.

The last day of the Congress was dedicated largely to issues surrounding testing of antiphospholipid antibodies. Ron Derksen's review of lupus anticoagulant testing was thorough and balanced. He noted that many have found that the presence of lupus anticoagulant correlates best with thrombosis. He cautioned, however, that each laboratory must establish its own reference ranges and use normalized ratios to express results. Tom Exner's presentation of procoagulant phospholipids interfering in tests for lupus anticoagulant proved very interesting. Several prominent investigators from Italy, Switzerland, France and Australia raised the continuing controversies and problems involved in antiphospholipid ELISAs, proposing international positive controls and more widely accepted guidelines for specimen collection and handling, kit manufacture, quality control and interpretation. The issue of assay specificity was the subject of much discussion. Dr. Derksen suggested subgrouping APS patients according to how many of the three tests (lupus anticoagulant, anticardiolipin and anti-beta2-glycoprotein I) are positive. Dr. Yasuda stressed the problems inherent in lupus anticoagulant testing. He then emphasized the strengths of his anti-phosphatidylserine–prothrombin assay and recommended its use to confirm lupus anticoagulant screening results.

On a sad note, Nigel Harris and Silvia Pierangeli reminded us that Azziz Gharavi had passed away in the fall of 2004. Azziz was a talented physician and investigator whose contributions to APS remain unmatched. He was not only crucial to the early development of an immunoassay for anticardiolipin antibodies, but he also made very significant contributions

to clinical aspects of APS. More recently, he studied murine models of the disease and made the first important discoveries regarding a possible infectious trigger for antiphospholipid antibodies. Azziz had a sharp wit and intellect and was a loyal, close friend to many of us—he will not be forgotten.

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