To the Editor:

Oosting et al's\(^1\) recent report on various phospholipid/protein antigen complexes (involving prothrombin, protein S, or protein C) recognized by antiphospholipid antibodies (aPL) has set the stage for other aPL investigators to redefine and evaluate the binding specificity of aPL. The nature of the neoepitopes induced by protein/phospholipid interactions is still unresolved. Firstly, the antigenicity of the phospholipid may be changed\(^2\) or, alternatively, cryptic epitopes may be expressed on the protein cofactors when associated with phospholipids.\(^3\) Shared epitopes formed between the protein and phospholipid could also be potentially involved in the binding reactivity of aPL.

Naturally occurring autoantibodies, including aPL, are increasingly found as a common feature in normal human sera (NHS)\(^4,5\). A proportion of natural aPL are "masked" in NHS, but can be shown and detected in enzyme-linked immunosorbent assay (ELISA) by prior heat-inactivation of the sera at 56°C for 30 minutes.\(^6\) These natural aPL may, under certain dysfunctions in immunoregulation, become pathogenetic.\(^4\) The aPL serum cofactor, β₂-glycoprotein I (β₂-GPI) has been shown to affect the binding of natural aPL differentially from its enhancing role for autoimmune aPL. In phospholipid ELISA, β₂-GPI, when complexed with the phospholipid, reduced the IgG aPL binding in NHS.\(^4,5\) We have now observed a similar differential effect of purified prothrombin on aPL binding. In cardiolipin phospholipid ELISA, the addition of prothrombin decreased the natural IgG aPL binding\(^6\) (Table 1). There was negligible binding of natural aPL to prothrombin alone. This indicates that the molecular presentation of prothrombin/phospholipid complex is altered, with resultant greater IgG aPL binding in autoimmune sera, but is unfavorable for natural IgG aPL binding.

| Table 1. Influence of Prothrombin on IgG Anticardiolipin Binding in ELISA |
|--------------------------|----------------|----------------|----------------|----------------|----------------|
|                         | Serum 1 | Serum 2 | Serum 3 | Serum 4 | Serum 5 |
| Prothrombin             | 7       | 8       | 6       | 8       | 6      |
| Prothrombin and cardiolipin | 56  | 66      | 43      | 65      | 58    |

Values are percentages of IgG binding relative to IgG binding to cardiolipin alone (100%). Twenty-five microliters per milliliter of prothrombin (12.5 µmol/mL; Sigma, St Louis, MO) was incubated with cardiolipin-coated ELISA wells for 30 minutes, after which a 1:25 dilution of normal human sera (heat-inactivated to "unmask" aPL) was added and the ELISA was processed in the usual manner. Modified and reprinted with permission from Cheng.\(^6\)

The meaning of this opposing influence of protein cofactors such as β₂-GP I and prothrombin on IgG aPL reactivity in normal and autoimmune sera is not known. This is reminiscent of the T-cell-derived suppressor/potentiating binding factors of IgE and IgA antibody binding described by Ishizaka\(^4\) and Tomasi,\(^9\) respectively. The regulatory roles of this dual function of aPL cofactors are certainly an important aspect of aPL immunobiology that needs to be unravelled.

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Differential binding of antiphospholipid autoantibody in normal and autoimmune sera to prothrombin/phospholipid antigen complex
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