mechanisms of these antibodies may provide new insight into APS pathogenesis that might ultimately suggest targeted approaches to therapy.

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Coming full circle with factor XIII

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The studies by Smith et al reported in this issue of Blood send us full circle, back to the starting model for the fibrinogen complex with plasma FXIII. These authors report that plasma factor XIII, called FXIII-A2B2, binds to the αC domain of fibrinogen with high affinity, ∼ 30nM. Factor XIII is a plasma- and platelet-derived protransglutaminase that is essential for hemoostasis and wound healing. Twenty-year-old studies show fibrinogen is a cofactor for the thrombin-catalyzed activation of FXIII-A2B2. In 1981, Lorand’s group reported that fibrinogen, and specifically a fragment from the fibrinogen α chain, modulates the generation of catalytically active factor XIII, FXIIIa. In 1982, Greenberg and Shuman showed FXIII-A2B2 bound specifically to fibrinogen with an affinity of ∼ 10nM. Based on this affinity, they concluded that fibrinogen and FXIII-A2B2 circulate as a complex in plasma.

Subsequent studies by Mosesson’s group support the existence of this circulating complex. They found FXIII-A2B2 copurifies with fibrinogen, but selectively with a fibrinogen fraction called peak 2. Further characterization showed pure FXIII-A2B2 mixed with pure fibrinogen coeluted on ion exchange or gel filtration chromatography as long as the fibrinogen component was peak 2, and not peak 1. In contrast, platelet factor XIII, FXIII-A2, did not coelute with either fibrinogen. The most substantive difference between peak 1 and peak 2 fibrinogens is the presence of the alternatively spliced variant fibrinogen chain known as γ'. This variant is 16 residues longer than the more common chain, called γA. Because fibrinogen is a dimer and the 2 chains are stochastically assembled, ∼ 15% of fibrinogen molecules are heterodimers with 1 copy of each variant. The authors concluded that interactions between γ' fibrinogen and the B subunit of FXIII-A2B2 support the circulating complex. The model shown in panel A of the figure is based on these studies.

Follow-up studies from Farrell’s group also suggested preferential binding to the γ'/γA heterodimer, but added 2 surprising findings. First, the difference in affinity between the 2 fibrinogen forms was only 20-fold; the heterodimer bound more tightly than the γA/γA homodimer. Because the concentrations of the 2 chains in fibrinogen differ by ∼ 15-fold, one would anticipate FXIII-A2B2 would distribute between the 2 forms and not be exclusively bound to peak 2 fibrinogen. Of note, the influence of calcium concentration differed between these 2 fibrinogen forms. Second, these studies showed the complex existed with a stoichiometry of 2:1, with 2 fibrinogen molecules for each FXIII-A2B2 molecule. As there are 2 B subunits, it appears reasonable that each FXIII-A2B2 molecule could bind 2 fibrinogen molecules. The model shown in panel B of the figure is based on these studies.

The current work characterized the interactions between factor XIII and fibrinogen, but focused on the fibrinogen αC residues 233–425. Using surface plasmon resonance to measure both kinetic and equilibrium parameters, they examined interactions between immobilized fibrinogen, fibrin, or recombinant fragments of the α chain and the 5 forms of factor XIII: (1) recombinant FXIII-A2, analogous to factor XIII found in platelets; (2) activated rFXIII-A2, (3) FXIII-A2B2 purified from plasma (4) FXIII-A2B2 cleaved with thrombin and assayed in the presence of EDTA, which limits subunit dissociation; and (5) activated FXIII-A2B2, cleaved with thrombin and assayed in the presence of calcium. No binding was detected with rFXIII-A2. After activation, rFXIII-A2 bound with moderate affinity to the fragment of α chain residues 371–425, Kd = 3pM. All 3 forms of FXIII-A2B2 bound to this α chain fragment with nanomolar affinity; Kd varied from 5nM (form 4) to 30nM (form 5). As the affinity for activated FXIII-A2B2 is markedly stronger than for activated FXIII-A2, these data indicate that the B subunit is the binding partner. Moreover, all 3 forms of FXIII-A2B2 bound to immobilized fibrinogen and fibrin with affinities similar to those found with the shorter α chain peptides. Indeed, the binding affinities were nanomolar (Kd = 3–35nM) for all 12 combinations of FXIII-A2B2 and fibrinogen, fibrin, or αC fragments. This similarity suggests that the interacting domain in fibrinogen is α chain residues 371–425 and the interacting subunit in FXIII-A2B2 is B. These data suggest the model shown in panel C of the figure.

As with all biochemical experiments, each of these studies has its limitations. Greenberg and Shuman immobilized fibrinogen on acrylonitrile beads and measured binding of radiolabeled factor XIII. As surface-bound...
fibrinogen is different from solution fibrinogen, these studies may not be relevant to solution complexes in plasma. Of note, the concern for surface immobilization is also true of the current studies. Here however, they also completed competitive inhibition studies and showed the α chain peptide was a competitive inhibitor of activated FXIII-A2B2 (that is, subunit B) binding to fibrin. The Mosesson studies are limited by the nature of peak 2. Peak 2 fibrinogen clearly contains γ′/γA heterodimers, but this peak may have other coincident differences as well. Perhaps the γ′ chain favors a conformation that exposes the relevant α chain domain. Lastly, the studies of Farrell’s group measured interactions by equilibrium analytical ultracentrifugation. This approach requires that FXIII-A2B2 and fibrinogen remain together at high concentrations for more than 24 hours. Under these conditions, Mosesson has found and we have confirmed, FXIII-A2B2 will catalyze the formation of γ dimers in fibrinogen. Thus, Farrell’s studies may measure the interaction of FXIII-A2B2 with fibrinogen dimers, leading to the observed 2:1 stoichiometry. We have recently examined the binding of FXIII-A2B2 to recombinant fibrinogens γA/γA, γA/γ′, and γ′/γ′. All 3 fibrinogens bound with equal affinity, $K_d = 41 \text{nM}$. These results suggest the γ′ chain is not critical for binding FXIII-A2B2. Nevertheless, because our studies were done with immobilized fibrinogens, the results may not be relevant to binding in solution.

In conclusion, the very clear and convincing data in the current studies support the original experiments that associated cofactor activity with the fibrinogen α chain. Nevertheless, further studies are needed to directly define the interactions in a solution complex between fibrinogen and factor XIII. Ongoing studies in my laboratory have not identified this complex in solution. It is notable that all of the published studies were completed in somewhat contrived conditions relative to circulating blood. Therefore, it remains an open question whether or not plasma fibrinogen is a carrier for FXIII-A2B2.

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