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A Rebuttal: Cross-linking of Fibrinogen by Factor XIII Zymogen Is Not Apparent in vivo

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A rebuttal: Cross-linking of fibrinogen by factor XIII zymogen is not apparent *in vivo*

Dear Sir,

Shainoff and DiBello have reasoned in their letter-to-the-editor that life would be very difficult if the factor XIII "zymogen" (A_2B_2) were enzymatically active *in vivo* without first having to be cleaved by thrombin to form XIIIa. This argument is based mainly upon the assertion that levels of crosslinked fibrinogen in plasma are normally very low, and that they would be much higher if factor XIII functioned as an enzyme. While not wishing to engage in a dispute on how much of such reaction products might or might not be expected, we did point out in our recent article that there is abundant evidence that crosslinking of fibrinogen does occur in circulation, albeit usually at low levels (1). We summarized the evidence in our article, including calling attention to the fact that α_2-antiplasmin is normally found covalently crosslinked to circulating fibrinogen (2). Shainoff and DiBello further believe that when crosslinking of fibrinogen occurs, the effects would more likely be due to the presence of thrombin-activated XIIIa or to tissue transglutaminase, or else be attributable to conformational changes in factor XIII occurring as a result of storage or freezing. There is, however, no evidence that such conformational changes occur. Be that as it may, failure to observe the effects of an enzyme's activity is not the same as proving that the putative enzyme has no potential for such activity. We emphasized in our article that the low level of observed activity is related to conditions that serve to suppress factor XIII activity, at least two of which were identified. We also provided irrefutable evidence that fibrin is a much better substrate for factor XIII than is fibrinogen, and almost as good a substrate as it is for factor XIIIa. We therefore suggested that the formation of cross linked fibrin in the circulation does not require concomitant activation of factor XIII by thrombin, although it certainly may occur. Thus, we suggested that factor XIII serves a role that heretofore has been exclusively ascribed to factor XIIIa and/or to tissue transglutaminase.

When we first made these observations on the enzymatic activity of factor XIII, like Shainoff and DiBello, we wondered whether the crosslinking we observed could be due to a conformational change brought about by the purification process and/or storage conditions. Our experiments employing casein and cadaverine as substrates and the experiments with plasma as the source of the factor XIII were designed to address this question. If processing and storage had somehow resulted in activation, one would have expected comparable levels of activity regardless of the substrates employed, something we did not observe. Instead, the crosslinking activity of factor XIII appears to be specific for fibrinogen, fibrin, and based upon our former studies, possibly also α_2-antiplasmin (2). We postulated that cooperative interactions between factor XIII and its native substrates brings about activity. X-ray crystallographic data support this postulate in that no large conformational change occurs in factor XIIIa upon thrombin cleavage (3).

Other experiments have permitted us to distinguish functionally between factor XIII and factor XIIIa. Pretreatment of factor XIII or plasma with N-ethylmaleimide (NEM), a sulfhydryl-reactive agent that irreversibly binds the active site sulfhydryl of factor XIIIa, failed to significantly inhibit the activity of factor XIII. In addition, we treated plasma with

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PMSF to inactivate any thrombin that might have been generated following calcification (1). Although Shainoff and DiBello have pointed out that PMSF is slow acting and short lived, it nevertheless appeared to be an adequate inhibitor in that thrombin activity, measured in terms of fibrinopeptide release or S2238 hydrolysis, was not detected, and visible clotting did not occur (1). If PMSF was omitted, clotting was evident within 30 minutes. We have subsequently repeated these experiments utilizing hirudin or PPACK as the thrombin inhibitor, and the results as far as fibrinogen or fibrin crosslinking are concerned have been the same.

Shainoff and DiBello emphasized that only cellular factor XIII (i.e., the A_2 dimer) can be activated by high salt conditions, and the failure to activate the plasma form of factor XIII (A_2B_2) with high salt is related to the association between A and B subunits (4). However, it is widely appreciated that the A_2B_2 complex can be dissociated at high calcium ion concentrations, with consequent non-proteolytic activation of XIII (5-7). What is even more remarkable about this calcium-dependent phenomenon is that the calcium level required for non-enzymatic activation of XIII is reduced to the physiological range in the presence of fibrinogen (6), an observation that clearly presaged our recent findings (1). In summary, we disagree with Shainoff and DiBello's notion that factor XIII cannot function as an enzyme in plasma, since we think that we have made a very good case for this. What is more important than disputing its potential activity is to continue to investigate the basis for the relatively low level of activity that occurs under physiological circumstances.

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High prevalence of prothrombin G20210A mutation among patients with deep venous thrombosis in Lebanon

Dear Sir,

Factor V Leiden is the most common genetic risk factor for deep venous thrombosis (DVT). We have previously shown a high prevalence of factor V Leiden mutation among healthy individuals and patients with DVT in Lebanon (1). This high frequency (40%), together with an exceptionally high prevalence of homozygous state (15%) suggest that the eastern Mediterranean region is probably the area of origin of this factor V Leiden mutation (1). Prothrombin G20210A mutation has been described as the second most frequent genetic risk factor for DVT with a reported frequency of heterozygotes of 4.8% (2). On the other hand, the geographic distribution of this 20210 G to A prothrombin variant among healthy individuals varied from 0.7 to 4.0% with an overall prevalence estimate of 2.0 % (3). This mutation is more frequent in southern Europe as compared to northern Europe and is very rare in Asia and Africa (3).

We tested 100 healthy Lebanese individuals from all Lebanese communities and regions and 40 consecutive Lebanese patients with DVT admitted to the American University of Beirut-Medical Center. Total genomic DNA was extracted from peripheral blood mononuclear cells after informed consent and

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