Activation of Factor X by Factor VIIa on Monocyte Cell Surfaces

To the Editor:

The recent report by Hoffman et al1 regarding the activation of factor X (FX) by factor VIIa (FVIIa) in the presence of monocytes with no cell-surface tissue factor argues for the presence of a monocyte surface-specific reaction that does not involve anionic phospholipid. They suggest that this, entirely novel, reaction is a contributory factor in the prevention of a bleeding syndrome after the administration of high doses of recombinant FVIIa to hemophiliacs. We take issue with their conclusion that this reaction is independent of anionic phospholipid and suggest experiments that would answer this question succinctly.

The investigators have clearly demonstrated inhibition of FXa/FVa-mediated prothrombin activation by prothrombin Gla domain but have not shown this to be independent of a protein-protein interaction. Inhibition by sequestration of phospholipid binding sites has been assumed despite clear evidence of a prothrombin fragment 1-FX interaction.2 We have recently reported studies on the FVII Gla domain, showing that inhibition in the FVIIa-mediated activation of FX is caused by Gla domain-protein interactions.3 We also demonstrated that FVIIa Gla domain binds to different (though not exclusively so) phospholipid motifs to FX. Inhibition of FX binding to phospholipid did occur but with a Kᵣ greater than 10 μmol/L, several orders of magnitude higher than both FXa activity inhibition and than the concentration of prothrombin Gla used by Hoffman et al.4 Their report showed no direct evidence that prothrombin Gla inhibits FX or FVIIa binding to phospholipid at the concentration used, but merely repeats the tacit assumption that this is so. Phospholipid binding studies using labeled proteins and defined phospholipid mixtures would establish whether prothrombin Gla does inhibit these interactions.

FX activation by FVIIa in the presence of anionic phospholipids and calcium ions but in the absence of tissue factor (TF) has previously been reported5 as occurring at approximately 40,000-fold slower than in the presence of TF. This difference, in conjunction with the difference between the binding affinities of FVIIa for phospholipid/TF or phospholipids alone, may explain the necessity for such a high dose of recombinant FVIIa in alleviation of a bleeding syndrome in hemophiliacs.

In summary, we would argue that Hoffman et al1 have failed to demonstrate conclusively that anionic phospholipid is not involved in the monocyte-enhanced activation of FX by FVIIa. We propose that the enhancement of FVIIa-mediated FX activation in the presence of monocytes is caused by the presence of suitable phospholipid motifs on the monocyte surface. These motifs, we would further surmise, are not expressed on endothelial cells. The activation of FX by FVIIa is caused by the assembly of a reactive complex on a specific phospholipid surface. This, along with the kinetic data for FVIIa, provides an adequate explanation for the physiologic effects observed.

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Response

In our recent report,1 we state that factor VIIa (FVIIa) activation of factor X (FX) is supported by monocytes through a tissue factor-independent mechanism. We speculated that this activation was dependent on a specific monocyte component in addition to the membrane phospholipid. This speculation was supported by three lines of evidence: (1) the activity was present on monocytes but not on a different cell type (endothelial cell line); (2) the activity was saturable with respect to FVIIa with a Kᵣ of 0.4 to 0.6 nM; and (3) the activity was not inhibited by the Gla domain of prothrombin. This additional component was not tissue factor, because anti-tissue factor antibodies did not inhibit the activity.

In their letter, Martin and Tuddenham agree with our major conclusion that the reaction is independent of tissue factor, but suggest that our data could be explained by the assembly of reactive complexes of FX and FVIIa on the specific anionic phospholipid surface provided by monocytes without invoking any additional component. The basis for their conclusion, in part, revolves around questions concerning our use of a Gla peptide as an inhibitor in our previous studies. We believe that our original conclusions are correct and provide additional data below.

Previous studies2,3 have shown that, on phospholipid surfaces, increasing concentrations of FVIIa give increasing rates of FX activation up to concentrations of 15 nM/L FVIIa. Because we saw saturation on monocytes with respect to FVIIa, either a monocyte component is limiting or the amount of phospholipid surface is limiting. In data not published in our original report, we have shown that monocytes will also support FXa activation of FX. However, this reaction did not saturate with increasing FIXa, implying that the phospholipid surface is not limiting.

Previous studies have shown that the Gla domain of bovine prothrombin can interact directly with a phospholipid surface.4 In addition, a number of studies have also shown that there are specific interactions between coagulation protein Gla domains.5 Whatever the mechanism behind its activity, a Gla peptide has different effects on purely phospholipid-dependent and cofactor-mediated activity of FVIIa. As shown in Fig 1, the Gla peptide from bovine prothrombin...
Fig 1. Effect of prothrombin 1-42 on FVIIa activation of FX. FVIIa (5 nmol/L) was incubated with factor X (plasma concentration of 170 nmol/L), phospholipid vesicles (40 μmol/L of 30% phosphatidylserine, 70% phosphatidylcholine), calcium (5 mmol/L), and the indicated concentration of Gla peptide from bovine prothrombin (1-42) in the presence (●) or the absence, (□) of tissue factor (2 nmol/L). At timed intervals, samples (10 µL) were removed and assayed for FXa (100 µL of 0.5 mmol/L Spectrozyme FXa, 1 mmol/L EDTA). Reactions without tissue factor were assayed every 10 minutes for 1 hour. Reactions with tissue factor were assayed every 30 seconds for 3 minutes. The rate of FX activation at 0 nmol/L Gla peptide was set to 1.

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Activation of factor X by factor VIIa on monocyte cell surfaces [letter; comment]

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