To the editor:

**FLT3 internal tandem duplication mutations are rare in agnogenic myeloid metaplasia**

We read with interest the recent article by Kelly et al.¹ that FLT3 internal tandem duplication (FLT3-ITD) mutations, while sufficient to induce a chronic myeloproliferative disease in a murine bone marrow transplant (BMT) assay, were not sufficient to induce an acute myeloid leukemia (AML) phenotype. These data suggest that FLT3-ITDs may require additional cooperating mutations to generate the AML phenotype. The animals developed a leukocytosis, a hyperplastic bone marrow with significant reticulin fibrosis and marked splenomegaly. The authors suggested that it would be worthwhile to investigate chronic myeloproliferative syndromes, including myeloid metaplasia with fibrosis, for the presence of activating mutations in FLT3. We report here our findings from the study of FLT3-ITD mutations in 40 patients with agnogenic myeloid metaplasia (AMM).

Genomic DNA was prepared from peripheral blood samples, following informed consent, from 40 patients with well-characterized AMM using the Nucleon Biosciences BACC II kit (Tepnel Life Sciences, Manchester, United Kingdom). All cases of AMM fulfilled the following criteria: a leukoerythroblastic blood picture, teardrop poikilocytosis, absence of monocytosis, marked bone marrow fibrosis, and lack of the Philadelphia chromosome. Patients with the closely related disorders, postpolycythemic myelofibrosis, and myelodysplasia with myelofibrosis were excluded from the study. DNA was screened by polymerase chain reaction (PCR) and conformation-sensitive gel electrophoresis (CSGE) for the reported FLT3 internal tandem duplication mutations, in exon 13 and 14, as previously described.²³ Positive control samples were included in the analyses (DNA from AML patients known to have FLT3-ITD mutations).

We did not find exon 13 or 14 FLT3-ITD mutations in the 40 cases of AMM studied, suggesting that such mutations do not play a significant pathogenetic role in the chronic phase of the disease. FLT3-ITD mutations, however, may emerge during transformation of MDS or at relapse of AML, suggesting that they promote leukemic progression.⁴⁻⁵ The lack of FLT3 mutations in AMM would support this conclusion, and it will be interesting to screen transformed patients to determine whether FLT3 mutations are involved in disease progression.

Faisal M. Abu-Duhier, Anne C. Goodeve, Gill A. Wilson, Rory S. Carr, Ian R. Peake, and John T. Reilly

Correspondence: J T Reilly, Academic Unit of Haematology, Royal Hallamshire Hospital, Sheffield S10 2JF, United Kingdom; e-mail: j.t.reilly@sheffield.ac.uk

References


To the editor:

**Platelet-dependent action of high-dose factor VIIa**

We read with interest the recent article by Butenas et al.¹ who conclude that the prohemostatic effect of high-dose factor VIIa in hemophilic blood or a synthetic plasma system is strictly dependent on tissue factor (TF). The authors note that their proposed mechanism differs from our previously published conclusion that high-dose factor VIIa can, in the absence of TF, generate factor Xa on the surface of activated platelets at concentrations sufficient to ameliorate hemophilia.²

Butenas et al propose that differences in the results from our 2 labs are due to differences in experimental design or protein preparations. We feel it is very likely that most of the differences in results between our 2 labs are due to our use of tissue factor–expressing cells as the initiating stimulus in our model system rather than tissue factor protein reconstituted into phospholipid vesicles. For example, Mann’s group has previously reported that the presence of zymogen factor VII in a synthetic plasma model competes with factor VIIa for binding to TF on phospholipid vesicles.³ By contrast, we see no effect of zymogen factor VII on thrombin generation in a model system initiated by cell-associated TF.⁴

Butenas et al suggest that high-dose factor VIIa therapy works in hemophilia to directly enhance activation of factor X through the factor VIIa/TF complex, resulting in an enhanced activation of platelets. Our data indicates that, although the factor VIIa/TF complex is essential to initiate coagulation, once platelets have been activated, high-dose factor VIIa binds to the surface of activated platelets and replaces the absent factor IXa/VIIIa complex to generate factor Xa, which then boosts platelet-surface thrombin generation.² Our view is consistent with the observation that the tissue factor pathway is intact in hemophilic patients²⁴ and, indeed, is responsible for platelet activation, accounting for the tendency of hemophiliacs to initially stop bleeding as a normal platelet plug forms⁵ but then suffer severe delayed rebleeding.

These disparate views of the mechanism of action of factor VIIa have important implications for dosing. The TF-dependent effect described by Butenas et al is saturated at levels below those now used therapeutically. By contrast, the binding of factor VIIa to the activated platelet surface is nowhere close to being saturated at therapeutically relevant concentrations. Thus, our mechanism
predicts that escalating the dose of factor VIIa should enhance its hemostatic effect, while the mechanism of Butenas et al suggests that escalating the dose would waste money without benefiting patients. Taken together, we believe the data supports a platelet-dependent, TF-independent mechanism for high-dose factor VIIa in hemophilia.

Maureane Hoffman, Dougald M. Monroe, and Harold R. Roberts

Correspondence: Dougald M. Monroe, UNC Chapel Hill, Hematology/Oncology, 932 Mary Ellen Jones Building, Chapel Hill, NC 27599-7035; e-mail: dmmorone@med.unc.edu.

References


Response:

Mechanism of factor VIIa–dependent coagulation in hemophilia blood

There are 3 areas in which the experimental results and their interpretations are in discord between our 2 groups: (1) whether factor VII and factor VIIa compete for tissue factor, (2) whether tissue factor is required for the function of factor VIIa in the condition of hemophilia A or B, and (3) whether vigorous propagation of thrombin generation by factor VIIa can occur on activated platelets in the absence of tissue factor, factor VIII, and factor IX. We are unsure of the basis for the discrepancies between the results. But we can offer the following facts in order to rationalize some potential bases for the differences:

First, the competition of factor VII and factor VIIa for tissue factor (TF) can only be observed at low tissue factor concentrations relative to the factor VII and factor VIIa concentrations. As the concentration of tissue factor is increased, the feedback activation of factor VII by factor VIIa–TF, factor Xa–membrane, and thrombin obliterates this phenomenon.1(Fig2)

Second, the tissue factor requirement essential to trigger the reaction appears to be indisputable. The relative rate of factor VIIa to factor VIIa–TF in factor X activation is approximately 0.0 001-fold,2 and the relative efficiency of factor VIII–factor IXa compared to factor VIIa–TF in factor X activation is approximately 50-100 to1.3 Our experimental data in synthetic system and in whole blood4 are consistent with these experimental ratios determined by other laboratories. During the propagation phase of thrombin generation, the rate of factor X activation by factor VIIa without tissue factor in the hemophilia A or B situation would be depressed by approximately 1 000 000-fold relative to that observed in normal blood in the presence of tissue factor.5

Third, the data of our recent publication4 indicate that factor VIIa at supraphysiologic concentrations has little effect on thrombin generation either in congenital hemophilia A or “acquired” hemophilia B blood. This observation is valid for experiments conducted in the absence of tissue factor and for those conducted at fixed TF concentrations. A prolonged clotting time in “acquired” hemophilia B blood (>40 min)$^{1(Fig4A)}$ and a lack of response to the anti-TF antibody in normal blood (see comments to Figure 2) indicate that the blood used in our experiments had no functional tissue factor. In contrast to the observation by Monroe et al,5 in our experiments thrombin generation in hemophilia blood is almost not affected by the initial rate of platelet activation. Data show$^{4(Fig4C)}$ that additions of 10 to 50 nM factor VIIa to “acquired” hemophilia B blood in the absence of tissue factor restores platelet activation to the levels observed in normal blood. But thrombin generation,$^{4(Fig4B)}$ however, is almost not affected by these factor VIIa additions. Similarly, while supraphysiologic concentrations of factor VIIa in congenital hemophilia A blood triggered with tissue factor lead to an increased rate of platelet activation,$^{4(Fig5D)}$ robust thrombin generation is not observed,$^{4(Fig5A)}$ even when platelets are completely activated. These observations would seem to contradict the notion that factor VIIa activity on platelets can provide for the rapid activation of factor X in the absence of the factor VIIa–factor IXa complex and tissue factor.

The experimental systems used by the Roberts group and our group differ in the source of tissue factor that is used to trigger the reaction. It is conceivable that the tissue factor source or the environment in which tissue factor is presented could be the cause of some of the differences in our results.

Kenneth G. Mann and Saulius Butenas

Correspondence: Kenneth G. Mann, Department of Biochemistry, University of Vermont, C–401 Given Building, 89 Beaumont Ave, Burlington, VT 05405

References


Platelet-dependent action of high-dose factor VIIa

Maureane Hoffman, Dougald M. Monroe and Harold R. Roberts