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and underwent a splenectomy resulting in a decreased but persistent red cell transfusion requirement. On referral, we found leukoerythroblastic peripheral blood morphology with dacrocytes, and his marrow also indicated PMF, without JAK2 or cMPL mutations.

Somatic *CALR* mutations have been identified in patients with ET and MF who lacked *JAK2*^{V617F} or *cMPL* mutations.[3,4] We detected a *CALR* 52-bp deletion type 1 mutation[3] in the granulocytes of both patients, but the CD19-positive sorted CLL cells had no detectable *CALR* mutation (Figure 1D-F). It is not known if *CALR* mutations in MF and ET are disease-originating mutations or more similar to the *JAK2*^{V617F}-positive MPNs, wherein other somatic or germ line mutations are thought to precede the MPN phenotypes.[5]

CALR mutations are reported to be associated with a lower risk in MPNs and thus are likely to be acquired earlier,[3,4] raising the possibility that preF-MF and CLL may arise from the same pluripotent stem cell. However, the lack of *CALR* mutation in these patients' CLL cells suggests that they represent independent clones. However, as the X chromosome–based clonality assays can be performed only in women, a common precursor origin of MPN and CLL clones cannot be completely excluded in these male patients.

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Response

Platelets do not generate activated factor XII—how inappropriate experimental models have led to misleading conclusions

Interestingly, for the research on the role of platelets in contact activation of coagulation, 2 articles on the topic using the same polyphosphate (polyP) preparation have been published.[1,2] In our paper in *Blood*, "Putting polyphosphates to the test: evidence against platelet-induced activation of factor XII,"[1] we found no evidence for platelet-induced activation of factor XII (FXII). Conversely, the paper in *Cell*[2] concluded the following: "PolyP represents the long sought 'foreign' surface that triggers fibrin formation by activated platelets linking primary to secondary hemostasis and critically contributing to 'procoagulant' platelet activity." Obviously, these 2 papers contradict each other, and it is useful for progress in the field to consider why. To support their position that platelet-derived polyP activates FXII, some of the authors of the *Cell* paper[2] tried to explain our negative findings in a letter to the editor.[3] Unfortunately, their letter contains statements based on factual errors and unfounded assumptions. Therefore, we wish to correct these errors and present possible explanations to the discrepancies that could be valuable for researchers in the field.

To support their claim that platelet-derived polyP activates FXII,[2] it is incorrectly assumed in the letter[3] that lack of FXII activation in our paper[1] can be explained by use of an old preparation and thus degraded polyphosphates. However, it is important to note that all activity measurements were performed within 1.5 months from receiving the substance donated by the Renné laboratory. It is also incorrectly stated that our findings were a result of using polytetrafluoroethylene filters, with the claim that such filters activate FXII. However, this claim is based on a citation that mentions neither contact activation nor FXII.[4] In fact, polytetrafluoroethylene is extensively used in blood-contacting biomaterials and shows very low procoagulant activity.[5] Regardless, polytetrafluoroethylene filters were not used in our paper. Figure 1A demonstrates that plasma filtration with the Minisart filters used[1] does not generate detectable FXIIa.

Their letter[3] further dismisses our results that were obtained using the sensitive fluorogenic substrate Boc-Gln-Gly-Arg-AMC (7-Amino-4-methylcoumarin) to measure FXIIa, claiming potential

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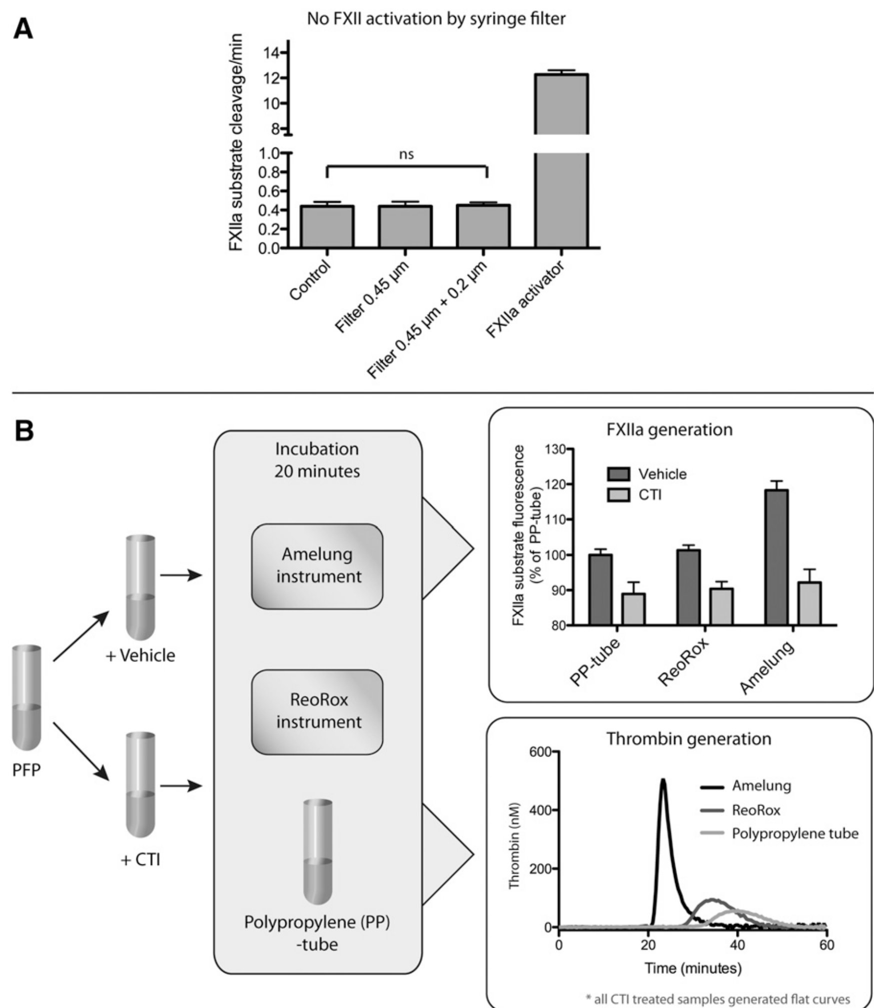

Figure 1. Contact activation in vitro. (A) Filtration with Minisart filters does not cause significant contact activation: FXIIa generation was measured before (control) and after filtration of plasma with the filters used in our *Blood* paper,[1] and kaolin (100 μg/mL) was used as positive control (n = 4, ns, nonsignificant Student *t* test). (B) Amelung KC4 causes contact activation, leading to substantial thrombin generation: pooled citrated plasma samples with or without CTI corn trypsin inhibitor (CTI) were incubated for 20 minutes at 37°C in Amelung KC4, ReoRox4, or polypropylene (PP) tubes. FXIIa generation was measured as described previously[1] with the fluorescent substrate present during incubation in the instrument. Bars represent FXIIa substrate fluorescence as percentage of the PP tubes (n = 8, background fluorescence of inhibitor/vehicle additions were compensated). Aliquots from incubated plasma were used for subsequent real-time thrombin generation measurements in the presence of 10 μM phospholipids. Curves represent means of 4 independent experiments.

unspecific cleavage by other proteases. However, when we measured FXIIa generation in citrated plasma with activated platelets or platelet-derived polyphosphate, we found little if any substrate cleavage compared with well-known contact activators.[1] Aristotelian logic tells us that, given the observations that A (FXIIa) and B (other proteases) give C (substrate cleavage), the deduction that absence of C necessarily entails the absence of A must hold true, regardless of potential activities of other proteases (B). Thus, the argument put forward is logically invalid. Additionally, FXII-deficient plasma or FXIIa inhibitor was always used as controls.

It is also claimed in the letter[3] that we are unable to reproduce our own data, citing an article where platelet agonists shortened clotting times more dramatically[6] than what was reported by us recently.[1] Crucially, however, they do not inform the reader that 0.6 pM tissue factor (TF) was added in the experiments reported in our *Blood* paper,[1] whereas no TF was added in the previous study.[6] Obviously, this difference in the conditions accounts for the discrepant results between the experiments.

Notably, there are no comments on our critique of their use of the Amelung instrument in their coagulation experiments. In fact, Figures 5D and S3A in their *Cell* paper[2] show that their coagulation assay is afflicted with artifactual contact activation, because "spontaneous coagulation" was significantly more rapid in normal than in FXII-deficient plasma. Recent publications have highlighted how low-grade contact activation is greatly amplified by the presence of phospholipids simulating the procoagulant membranes of activated platelets.[7,8] Thus, the dramatic effects on recalcification times observed with preactivation of platelets in their study[2] likely stem from platelet-dependent amplification of material-induced contact activation and not from FXIIa generation by platelets per se. In Figure 1B, we show that the Amelung instrument they used[2] generates far more FXIIa than comparable assays. Incubating plasma in the Amelung generates sufficient FXIIa to induce robust thrombin generation in the presence of phospholipids, indicating that artifactual contact activation likely affects clotting times in this instrument. In this debate, it is essential to recognize that any kind of material can induce some degree of FXII activation, including plastics. In fact, this is also demonstrated in Figure 1B in their letter,[3] where normal plasma without activator still causes thrombin generation compared with the flat curve in FXII-deficient plasma.

To minimize the detrimental effects of material-induced contact activation, we used 0.6 pM TF in one of our experiments (Figure 4 of our *Blood* paper).[1] The letter claims that the addition of TF conceals the effects of platelet activation and platelet PolyP on coagulation times, basing these conclusions on thrombin generation measurements in plasma.[3] However, the differences in clotting times caused by the use of different TF/liposome preparations and instruments with varying degree of contact activation potential (Figure 1B) makes direct comparisons between the experiments impossible. Indeed, in Figure S2B in our *Blood* paper,[1] we demonstrate that the ReoRox

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instrument used in our study is superior for detecting the effects of contact activation on coagulation compared with the Amelung, even when 0.6 pM TF was used with ReoRox compared with no TF in the Amelung.

Most regrettably, no mention was made in their letter[3] of the questions regarding the experiment wherein infusion of thrombin receptor activating peptide-6 (Trap-6) induced death by pulmonary embolism in 13 of 15 mice.[2] When repeating this pivotal experiment, we found no signs of pulmonary embolism or circulatory distress in challenged mice, which seems more reasonable, as PAR1, the receptor responsible for platelet activation by Trap-6, is not expressed by murine platelets. For the field to trust their *Cell* paper,[2] it is important that the authors can explain their results with Trap-6.

We want to emphasize that our results do not dispute that platelet polyP may play a role in coagulation in vivo, as was recently suggested in an article on inositol hexakisphosphate kinase 1 knockout mice.[9] However, that paper contains no evidence that the reported hemostasis defects are connected to FXII. Likewise, we do not exclude a role for FXII in thrombosis in vivo. However, we hold that currently there is no valid evidence to support a direct cause-effect relationship between platelets and the physiologically relevant activation of FXII.

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