enhanced secretion seen in PKCθ$^{-/-}$ platelets, and Vθ1-1-TAT had no additional effect in PKCθ$^{-/-}$ platelets. TAT alone had no effect. Moreover, we found that Vθ1-1-TAT significantly enhanced CRP-induced granule secretion in human platelets (Figure 1C), prepared in the manner described by Nagy et al.[4]

Together, our data suggest that PKCθ negatively regulates CRP-induced ATP secretion in human and mouse platelets, consistent with our reported increased thrombus formation under flow over collagen in vitro.[3] It is difficult to explain the differences between our data and those of Nagy et al[4] However, we note that Vθ1-1-TAT inhibited CRP-induced syntaxin 4 phosphorylation in human platelets, as shown in Figure 4 of Nagy et al.[4] Syntaxin 4 phosphorylation is expected to reduce its association with SNAP-23.[5] However, interaction between SNAP and syntaxin is necessary for granule secretion.[6-8] We would therefore expect that reduced syntaxin phosphorylation would lead to enhanced syntaxin-SNAP interaction and so enhanced secretion.

In conclusion, contrary to Nagy et al, we suggest that PKCθ negatively regulates CRP-induced dense granule secretion, although clearly there remains much to discover about the roles of PKC isoforms in platelet activation and thrombus formation.

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Contribution: M.T.H. designed and performed experiments, analyzed results and cowrote the manuscript. A.W.P. designed experiments and cowrote the manuscript.

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Response

Role of PKCθ in collagen-related peptide-induced platelet activation

Harper and Poole report that collagen-related peptide (CRP) caused potentiation of dense granule secretion in murine platelets deficient in Protein kinase C (PKC) θ isoform.[1] They also show that pretreatment of human platelets with PKCθ isoform selective RACK peptide results in enhanced dense granule secretion upon stimulation with CRP.[1] They argue that these results do not agree with our recent work[2] demonstrating a positive role for PKCθ in platelet activation.

Our experiments[2] were done with higher concentrations of CRP (10 and 20 μg/mL). It is evident from Figure 1 of the work by Harper and Poole[1] that the potentiating effects of CRP on dense granule secretion are more apparent at lower agonist concentrations and there is no potentiation when 10 μg/mL CRP was used. Hence, we decided to perform additional experiments with lower concentrations of CRP (2 and 5 μg/mL), which were not used in our previous study.[2] The lower concentrations of CRP (2 μg/mL) caused a potentiating effect on dense granule secretion from PKCθ$^{-/-}$ platelets (0.320 ± 0.0064 nmoles/3.7 × 10^8 platelets; $P < .05$), compared with wild-type (WT) littermates (0.238 ± 0.004 nmoles/3.7 × 10^8 platelets). Slightly higher concentrations (5 μg/mL) showed no significant differences (0.685 ± 0.044 vs 0.512 ± 0.044 nmoles/3.7 × 10^8 platelets; $P > .1$). Hence, we agree with Harper and Poole[1] that very low concentrations of CRP cause potentiation of dense granule release in platelets lacking functional PKCθ.

Harper and Poole[1] claimed that they reported that CRP-induced secretion is enhanced in their previous study.[3] In their previous study[3] they actually stated that "Interestingly, however, no difference in adenosine triphosphate (ATP) secretion was seen between PKCθ$^{-/-}$ and WT platelets in response to CRP (Figure 3B) or collagen (Figure 3C).[1] It is not clear to us how, using the same concentrations of CRP used in their previous study (1 and 5 μg/mL),[3] they could demonstrate significant potentiation in PKCθ null mouse platelets ($P < .001$) in Figure 1A of their study.[1] Hence their current results[1] are in conflict with their own previous work.[3] It is also important to note that in their previous study,[3] the ATP release numbers were nearly 10-fold higher than in the current work (Figure 1A[1]).

Consistent with our study,[2] Cohen et al[4] recently reported that PKCθ plays a positive regulatory role in platelet activation and secretion. Consistent with our in vivo thrombosis results,[2] they noticed that the bleeding times were increased in PKCθ$^{-/-}$ mice.[4]

Harper and Poole[1] argue that phosphorylated syntaxin 4 would dissociate from SNAP23 and thereby reduce secretion, thus syntaxin 4 phosphorylation would negatively regulate release. Previous studies[5,6] reported dissociation of syntaxin 4 and SNAP23 upon platelet activation though no time course for this process was given. Dissociation could occur after fusion, during which the components of the SNARE complex might be recycled, though it is

not clear whether this is significant in an activated platelet. The evidence that panPKC inhibitors abolish phosphorylation of syntaxin as well as secretion is indicative that syntaxin phosphorylation could be important for secretion.[5]

In conclusion, we emphasize that our data[2] are supported by a recent independent study[4] that confirms a positive regulatory role for PKCθ in platelets.

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Contribution: S.P.K. wrote the response and provided overall supervision and interpretation of the data; and J.J. performed the experiments.

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To the editor:

β1-tubulin gene mutation platelets are not macrothrombocytes

The brief report by Kunishima et al[1] describing a mutation of the β1 tubulin gene affecting microtubule assembly is of interest, but data presented do not support association of the genetic defect with macrothrombocytopenia. The authors stated both the male propositus and his mother had prominent appearance of giant platelets on peripheral blood smears, and provided light (Figure 1A[1]) and electron micrographs (Figure 1B[1]) to support that finding. The patient platelet in Figure 1A[1] has damaged peripheral cytoplasm that may make it appear larger than a normal cell, but, more important, the platelet is significantly smaller than any red blood cell in the same field. No discoid platelets from the control or patient are present in the electron micrographs (Figure 1B[1]). As a result, it is difficult to be certain there is any real size difference between patient and control platelets. Surely the authors must be aware that giant platelets from all of the known macrothrombocytopenias are larger than red blood cells, and many exceed the size of lymphocytes, monocytes, and neutrophils.[2] It might have been helpful if the authors had provided the mean platelet volumes of the mother and child.

The immunofluorescence studies raise 2 questions. Patient and control microtubule coils in the report's Figure 2A[1] are similar in size and appear to be perfect coils. If the patients had complete platelet microtubule coils, those coils should be many times the diameter of control coils. More important, giant platelets from the known inherited macrothrombocytopenias rarely have complete microtubule coils lying just under the surface membrane. Rather, the giant platelets are spherical in form, and their microtubules are often organized in a manner resembling balls of yarn.[3] The studies of the mutation in the present study are of interest. However, the statement "W318 β1 tubulin may interfere with normal platelet production, resulting in macrothrombocytopenia"[1] is not supported by the data presented.

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Response

W318 β1-tubulin and macrothrombocytopenia

We would like to thank Dr White for valuable comments on our study that reported the first human β1-tubulin mutation associated with congenital macrothrombocytopenia.[1] We have been working on congenital macrothrombocytopenia and analyzed more than 200 cases. We do not think that giant platelets from macrothrombocytopenia syndromes are necessarily larger than red blood cells. The mean platelet sizes