To the Editor:

I have read with interest the article by Camire et al\(^1\) proposing "endocytosis by megakaryocytes as the major mechanism by which platelet-derived factor V is acquired. . . ."

The approach is clever, identifying the type of factor V derived from two patients, each heterozygous for factor V Leiden, who received allogeneic transplantation of bone marrow and liver, respectively, from a donor with wild-type factor V.

The only data presented in detail is a series of four Western blots and, while the patterns exhibited are consistent with the hypothesis, this technique does not lend itself well to quantitative evaluation. In fact, the conclusion is based in part on the failure to demonstrate "data not shown." Moreover, the authors did not attempt to show that megakaryocytes can endocytose factor V. Further, the possibility that the result of the transplant is a chimera is not considered in detail.

Against this proposition are two reports from my laboratory indicating that factor V can be biosynthesized in guinea pig platelets\(^2\) and in human megakaryocytes\(^3\) performed by the incorporation of radiolabeled amino acids into factor V. A third report from our group, in collaboration with Alan Gewirtz, indicates that mature human megakaryocytes bind and synthesize factor V and have mRNA for factor V.\(^4\) Thus, the question is not whether there is biosynthesis, but whether uptake or synthesis is the "major" mechanism.

In a recent study from the laboratory of Ginsburg\(^5\) using factor V "knockout" mice transplanted with marrow progenitor cells, different cellular origins for the biosynthesis of murine plasma factor V and murine platelet factor V were demonstrated, suggesting that free interchange does not take place in a situation in which greater than 80% engraftment occurs. Because the differences between mice, guinea pigs, and humans could be caused by true biological variability, this study supports, but does not prove, that platelet factor V derives from megakaryocytes. Nevertheless, Camire et al have not excluded an important contribution to platelet factor V from megakaryocyte biosynthesis because they have not adequately addressed the effect of bone marrow chimeraism on their results, and have perhaps overestimated the ability of Western blotting to quantify the concentration of factor V.

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We thank Dr Colman for his interest in our study and appreciate the opportunity to respond to his thoughtful comments. He raises three major points. First, it should be noted that important cross-species differences have been demonstrated in the coagulation system (eg, unlike human platelets, which express PAR1, mouse platelets express PAR31). In particular, Dr Colman refers to data in his past publication3 and a recent abstract from the Ginsburg laboratory2 regarding guinea pigs and mice, respectively, that support megakaryocyte synthesis of factor V in those two species. However, those observations should be contrasted to our previous work in the bovine system where platelet-derived factor V contributes very little to the total blood factor V pool,4 arguing against significant levels of synthesis or uptake. Further, aortic endothelial cell synthesis of factor V has been shown in the bovine system, arguing against significant levels of synthesis or uptake. Further, aortic endothelial cell synthesis of factor V has been shown in the bovine system,2 in contrast to the human system.6 Given this spread of results, we believe animal models must be viewed with caution when interpreting the different mechanisms by which factor V accumulates in any tissue bed.

Our second point addresses the quantitative assessment of Western blots. Western blotting of factor V has been done in our laboratory7 as well as by others in our institution,5-10 routinely and quantitatively, over the last several years. Based on previously published data with samples from individuals homozygous for factor V Leiden,11 we estimate our lower level of detection for factor V Leiden, based on the appearance of its unique activated protein C cleavage products (54- and 60-kD bands), to be 2 to 3 ng/lane under the conditions described in our current report.12 In fact, as noted in that paper, we overexposed the Western blot of patient FW by fivefold to make certain we had the opportunity to detect even small amounts of platelet factor V Leiden which would have arisen because of megakaryocyte synthesis. Under those conditions we also failed to detect any specific bands at 54 and 60 kD. Thus, while we agree with Dr Colman that we cannot entirely rule out megakaryocyte synthesis of factor V, based on our collective data, it is unlikely that such production, if present, could account for more than 5% of the secretable platelet-derived factor V.

Finally, regarding the possible chimeric nature of our patients, polymerase chain reaction (PCR) (35 cycles) of DNA extracted from the peripheral blood cells of JMW posttransplant failed to reveal the presence of an FV Leiden allele due to retention of her original marrow. Because we were concerned about total marrow ablative pretransplant, we believed PCR analysis would be an exquisitely sensitive method to demonstrate lack of chimerism. Furthermore, because this transplant was male into female, marrow recovery was assessed by XY chromosomal cytogenetic display in phytohemagglutinin-stimulated marrow cells. JMW is now 84% XY, 0.2% XX, and 15% XO. The 15% XO cells are most likely XY (donor) that lost their Y, because additional analyses of the 55-year-old donor indicated he had lost his Y in several cells. While we cannot be certain that the XO were not in fact XX (recipient) that lost an X, this latter scenario is very unlikely, but still formally possible. Therefore, JMW is at least 84% donor blood cells, and most likely 99% based on the argument detailed above. These data coupled with the PCR analyses strongly argue for little, if any, chimerism.

Inadvertent bone marrow transplantation in liver transplants, without evidence of graft-versus-host disease, has not been documented. FW had no clinical evidence of graft-versus-host disease; hence, chimerism is very unlikely. Furthermore, no FV Leiden protein was detectable in her platelets and PCR analyses of DNA extracted from her peripheral blood cells posttransplant indicated a heterozygous FV Leiden genotype. Because of the undetectable level of factor V Leiden in FW’s platelets, if any chimerism were present, the donor megakaryocytes would be the only ones producing platelets. Such a scenario is highly improbable.

Consequently, we maintain that the most plausible interpretation of our collective data is that endocytosis of plasma factor V, most likely by the megakaryocyte, is the primary mechanism by which secreted factor V accumulates in human platelets. In both our patients, the phenotype of their secretable platelet factor V mirrored that of their plasma factor V, regardless of the factor V genotype present in their hematopoietic cells.

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Where Does Platelet Factor V Originate?

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