Comment on Pula et al, page 4035

Uncovering the dark side of PKCδ

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In this issue of Blood, Pula and colleagues provide a novel mechanism for the negative regulatory role of platelet protein kinase Cδ (PKCδ) that is independent of inside-out signaling, granular secretion, or early steps of GPVI signaling.

The initial step of agonist-induced platelet activation is platelet shape change, which is associated with intracellular calcium rise, phosphorylation of pleckstrin by PKC, and myosin light chain (MLC) by MLC kinase, followed by cytoskeletal rearrangement. Human platelets predominantly express 4 of the 12 known PKC isoforms. Although PKCα, β, and δ have been shown to positively regulate platelet activation, PKCδ is unique in that it plays a positive as well as a negative regulatory role.

In this issue, Pula and colleagues provide convincing evidence regarding the negative role of PKCδ in platelet aggregation and reveal a novel mechanism for regulation of actin and filopodia. Filopodia are membranous protrusions formed and supported by bundles of actin filaments and are followed by lamellipodia formation leading to platelet spreading.

Vasodilator-stimulated phosphoprotein (VASP) regulates actin polymerization and hence filopodia formation primarily through its antagonicking activity. VASP is a major substrate of protein kinase A, protein kinase G, and PKC, which phosphorylate it on Ser157, Ser239, and Thr278. Phosphorylation of VASP on Ser157 is required for its antagonicking activity.

In previous studies using pharmacological agents, it was suggested that PKCδ negatively regulates collagen-induced dense granule secretion. Using PKCδ knockout mice, Pula and colleagues show that negative regulation of platelet aggregation by PKCδ is independent of inside-out signaling, dense granule secretion, and early GPVI signaling. They also provide evidence that PKCδ physically interacts with VASP and inhibits VASP phosphorylation on Ser157 by classical PKC (cPKC) isoforms, thus suppressing actin polymerization and filopodia formation. The fact that cPKCs’ ability to phosphorylate other platelet proteins is unaffected by the inhibition or absence of PKCδ suggests that PKCδ does not directly inhibit the activity of these enzymes. This work undoubtedly provides provocative evidence regarding the negative role of PKCδ in platelet aggregation.
The characteristic biochemical lesion in paroxysmal nocturnal hemoglobinuria (PNH) is the clonal lack of glycosyl phosphatidylinositol (GPI)–linked proteins on the membrane of the affected blood cells. This lesion accounts for many of the clinical manifestations of the disease, particularly intravascular hemolysis and its consequences and, probably, the marked propensity for venous thromboses. The lesion results from mutations of the gene, called PIGA, that codes for one of the components necessary for the biosynthesis of the GPI anchor.

Hematopoietic precursors with defects in this gene have been identified in many if not most healthy donors tested, so it is clear that this mutation is not enough to account for the occurrence of the disease. The PNH clone must be selected and expanded in order for a sufficient number of blood cells to be present to cause clinical manifestations. It has been suggested that the clone is selected for by the immunologic processes underlying aplastic anemia (the Luzzatto-Young hypothesis) and, indeed, a large proportion of patients with aplastic anemia exhibit the cells characteristic of PNH, albeit usually in relatively small proportions. In only a minority of such patients does the clone expand to clinical relevance. Thus, neither the characteristic biochemical abnormality nor the selection process explains requisite expansion of the clone in those patients with PNH.

In their paper, Inoue and colleagues offer a possible insight into this problem. In 2 patients, the authors have found a rearrangement of chromosome 12 that has apparently induced a mutation causing the “deregulation” of the HMGA2 gene. This gene produces a protein, a member of the high-mobility group of proteins, that functions as an architectural transcription factor, promoting transcription by facilitation of the assembly of transcription factors into an “enhancosome.” Mutations causing deregulation of the gene have been found in benign mesenchymal tumors. Inoue et al argue that in these 2 patients, overactivity of the HMGA2 gene as a consequence of the chromosomal break gives the selected PIGA–mutated clone the expansion that it needs to become clinically manifest but not the uncontrolled expansion of a malignancy. Thus, PNH is viewed as a benign tumor of the bone marrow involving the selected GPI-deficient clone.

This paper’s importance is that it points to an area of study that may solve one of the 2 basic problems in understanding PNH: the basis of clonal selection and the basis of clonal expansion. Most patients do not have chromosomal abnormalities, and it will be difficult to know where to look for second mutations that

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### How many mutations does it take to get PNH?

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One of the puzzles in understanding paroxysmal nocturnal hemoglobinuria is the reason for the expansion of the defective clone. In this issue, Inoue and colleagues suggest that a second mutation might be needed.

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