IMMUNOBIOLOGY

Comment on Bartz et al, page 4102

Career choices of monocytes in dangerous times

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Monocytes can differentiate into a number of end-stage effector cells. In this issue of *Blood*, Bartz and colleagues show that danger signals via toll-like receptors (TLRs) drive monocytes to differentiate into macrophages and prevent them from becoming dendritic cells, through the up-regulation of suppressor of cytokine signaling (SOCS) proteins.

Cells of the innate immune system recognize infectious organisms through pattern recognition receptors that include members of the TLR family. Many hematopoietic progenitors, including hematopoietic stem cells, express TLRs, and their ligation can direct the differentiation of these cells into various lineages.[1] As precursor cells in the innate immune system, monocytes are of particular interest in this regard: they can differentiate into phagocytic macrophages, an important immediate defense against bacteria, or into dendritic cells (DCs), the most potent professional antigen-presenting cells that orchestrate adaptive immune responses. This switch may play an important role in host defense. For example, monocytes from patients with progressive, systemic leprosy become primarily macrophages, whereas monocytes from patients with limited disease become DCs as well as macrophages.[2]

Cytokines, particularly granulocyte-macrophage colony-stimulating factor (GM-CSF), are key drivers of the developmental switch of monocytes into DCs. The study by Bartz and colleagues offers mechanistic insights into this switch, showing that TLR signals override GM-CSF signaling and prevent DC maturation. This switch appears to operate through the induction of SOCS family proteins, which are increased after TLR ligation. Overexpression of SOCS1 also blocks GM-CSF signaling and prevents DC maturation.

There are a few important caveats to these conclusions. This work was performed in vitro, and there is no direct evidence yet that this mechanism pertains in vivo. Forced overexpression of SOCS proteins may have nonspecific effects that could limit the interpretation of these results. But the findings suggest a mechanism of how danger signals[3] influence the functional development of immune cells for short-term versus long-term defense. During an ongoing infection, microbial products alert the immune system to immediate danger, increasing the number of macrophages that can phagocytose the invaders, at the expense of DCs. This mechanism could help explain why patients with sepsis are immunodeficient, and may suggest differential strategies for modulating or amplifying immune responses depending on whether the immune system is responding to an active infection or preparing for a future one.

The author declares no competing financial interests. ■

REFERENCES

1. Nagai Y, Garrett KP, Ohta S, et al. Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment. Immunity. 2006;24:801-812.

2. Krutzik SR, Tan B, Li H, et al. TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cells. Nat Med. 2005;11:653-660.

3. Matzinger P. The danger model: a renewed sense of self. Science. 2002;296:301-305.

HEMOSTASIS

Comment on Pula et al, page 4035

Uncovering the dark side of PKCδ

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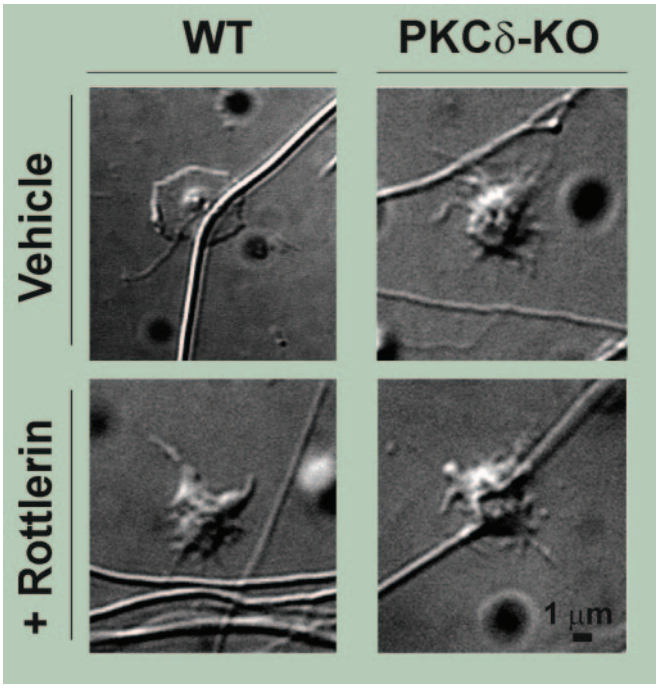
In this issue of *Blood*, Pula and colleagues provide a novel mechanism for the negative regulatory role of platelet protein kinase C δ (PKCδ) that is independent of inside-out signaling, granular secretion, or early steps of GPVI signaling.

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.[1] Although PKCα, β, and θ have been shown to positively regulate platelet activation, PKCδ is unique in that it plays a positive as well as a negative regulatory role.[2,3]

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.[4] Vasodilator-stimulated phosphoprotein (VASP) regulates actin polymerization and hence filopodia formation primarily through its anticapping 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 anticapping activity.[5]

In previous studies using pharmacological agents, it was suggested that PKCδ negatively regulates collagen-induced dense granule secretion.[3] 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 workundoubtedly provides provocative



PKCδ negatively regulates filopodia formation in human platelets. See the complete figure in the article beginning on page 4035.

sponsible for decreased VASP phosphorylation on Ser157, and (5) whether PKCδ is constitutively associated with VASP in resting platelets or whether it becomes associated upon stimulation of platelets will be very interesting. Thus, this work, using a genetic approach, provides an intriguing avenue for further studies that might aid in design and development of novel therapeutic agents for the treatment of thrombotic disorders.

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new ideas and will inspire new studies investigating how association of VASP and PKCδ inhibit phosphorylation of VASP on Ser157. The evidence presented strongly suggests that the PKCδ activity is necessary for the observed negative regulation.

Future studies aimed at (1) the stoichiometry of PKCδ interaction with VASP, (2) how PKCδ activity is involved in inhibiting filopodia formation, (3) whether PKCδ activation is required for its interaction with VASP, (4) whether steric hindrance or conformational change resulting from PKCδ binding is re-

REFERENCES

1. Jaken S. Protein kinase C isozymes and substrates. Curr Opin Cell Biol. 1996;8:168-173.

2. Moussazadeh M, Haimovich B. Protein kinase C-delta activation and tyrosine phosphorylation in platelets. FEBS Lett. 1998;438:225-230.

3. Murugappan S, Tuluc F, Dorsam RT, Shankar H, Kunapuli SP. Differential role of protein kinase C delta isoform in agonist-induced dense granule secretion in human platelets. J Biol Chem. 2004;279:2360-2367.

4. Naik UP, Naik MU. Association of CIB with GPIIb/IIIa during outside-in signaling is required for platelet spreading on fibrinogen. Blood. 2003;102:1355-1362.

5. Barzik M, Kotova TI, Higgs HN, et al. Ena/VASP proteins enhance actin polymerization in the presence of barbed end capping proteins. J Biol Chem. 2005;280:28653-28662.

RED CELLS

Comment on Inoue et al, page 4232

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.

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 "enhanceosome." 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