have more marked increases in release of potentially pruritogenic cytokines. Finally, through use of the MPN mutation analysis, they demonstrate that mast cells from MPN patients are indeed offspring of the malignant clone, potentially explaining their functional differences from normal mast cells. These observations identify an intriguing target for therapy among MPN patients with significant pruritus, and further expand our knowledge of functional changes in leukocytes seen in MPN patients.

There is now accumulating evidence that mast cells, basophils, and even platelets may all play a role in MPN pruritus. Intriguingly, preliminary reports of JAK2 inhibitor therapy report significant decreases in pruritus,[7] and we can be hopeful that further discoveries regarding the pathogenesis of MPN pruritus may yield additional insight into the overall pathogenesis of MPNs.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● PHAGOCYTES & GRANULOCYTES

Comment on Lämmermann et al, page 5703

Close encounters of the 3D kind

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The small GTPase Cdc42 is a key regulator of cell polarity. In this issue of *Blood*, Lämmermann and colleagues show that DCs without Cdc42 are still able to migrate fairly efficiently on 2-dimensional surfaces but become irreversibly entangled in 3-dimensional environments, both in vitro and in vivo.

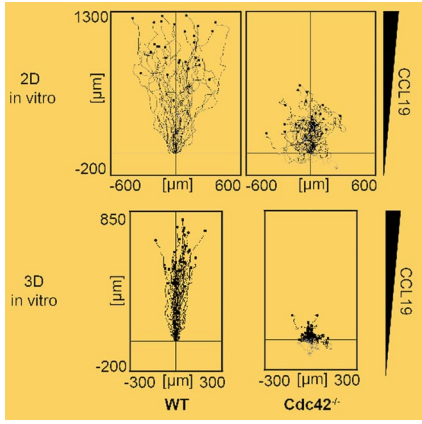
Dendritic cells (DCs) are critical for the initiation of adaptive immune responses by taking up antigen in the periphery, such as skin, to present it to lymphocytes passing through draining peripheral lymph nodes (PLNs). To perform this task efficiently, activated DCs switch their sessile sampling behavior to a highly migratory one, characterized by the acquisition of a polarized phenotype and increased expression of the chemokine receptor CCR7, which responds to its ligands CCL19 and CCL21.[1] These changes are prerequisites for efficient DC migration into afferent lymphatic vessels, which secrete CCR7 ligands and serve as a communication highway to draining PLNs.[1]

Small GTPases of the Rho and Ras families are key components of the induction and maintenance of a polarized phenotype and migration. Rac and Rho are involved in lamellipodia formation and uropod retraction, respectively. The Rho family member Cdc42 plays a role in induction and maintenance of polarity in various cell types, such as neutrophils and macrophages, in part through stabilization of the leading edge lamellipodia.[2] It remains unclear, however, how Cdc42 affected DC motility.

In this issue of *Blood*, Lämmermann et al report their findings on the role of Cdc42 during physiologic DC migration obtained in a series of elegant in vitro and in vivo assays.[3] Using primary mouse DCs derived from Cdc42-deficient bone marrow cultures, the authors investigate the migratory properties of these cells on 2-dimensional surfaces. Despite defects in maintaining polarity, Cdc42-deficient DCs still managed to migrate toward an increasing concentration of CCL19, with only slightly reduced migration velocities as compared with wild-type DCs (see top panel of figure). The residual migratory capacity was likely due to largely intact Rac-induced spreading and lamellipodia formation. Thus, on 2-dimensional surfaces, Cdc42 was not absolutely required for directed cell motility.

In a second set of experiments, Lämmermann et al examine the importance of Cdc42 during DC migration in geometrically more complex environments, that is, the 3-dimensional fibrillar networks of collagen matrices in vitro and dermis in vivo. Somewhat unexpectedly, DCs lacking Cdc42 were strongly impaired in their directed motility in 3-dimensional environments in vitro (see bottom panel of figure). Similarly, Cdc42-deficient DCs were entirely blocked in their migration from skin to draining PLNs, due to impaired entry into afferent lymphatic vessels. A more detailed morphologic analysis of Cdc42-deficient DCs uncovered that these cells became rapidly entangled within the 3-dimensional meshwork, with multiple protrusions pulling in different directions. Therefore, whereas migration efficiency in absence of Cdc42 was partially rescued due to the "lack of alternative routes" on 2-dimensional surfaces, cell motility in 3-dimensional environments absolutely required Cdc42.

Although the function of Cdc42 in other leukocytes was not addressed in this study, recent studies provide solid evidence for a



Single cell trajectories of wild-type (WT) and Cdc42-deficient DCs migrating along a CCL19 gradient in 2-dimensional (top panel) and 3-dimensional (bottom panel) settings. Despite reduced directionality, Cdc42-deficient DCs were still able to migrate with residual efficiency on 2-dimensional surfaces. In contrast, lack of Cdc42 dramatically reduced cell displacement in 3-dimensional environments due to irreversible cell entangling. See the complete figure in the article beginning on page 5703.

common integrin-independent, actin protrusion-dependent "amoeboid" migratory phenotype inside 3-dimensional environments in all hematopoietic cells.[4-6] The data presented by Lämmermann et al support the notion that directional "decisiveness" conferred by Cdc42 is a critical element of this migration mode in complex 3-dimensional settings. The observations may also explain the reduced migration of DCs deficient in the Cdc42-effector Wiskott-Aldrich syndrome protein,[7] although the more severe phenotype of Cdc42-deficient DCs reported by Lämmermann et al suggests the involvement of additional downstream effectors. Similarly, lymphocytes expressing a mutated form of the actin regulator Coronin1A, which results in excessive lamellipodia formation, show strongly impaired parenchymal motility.[8] Together with this latest report from Lämmermann et al, these findings highlight the importance of tightly controlling actin cytoskeletal dynamics for efficient "decision-making" and maneuvering through complex 3-dimensional pore systems.

In conclusion, the authors demonstrate a requirement for Cdc42 in 3-dimensional environments to avoid "cellular trapping." Their findings also highlight the importance of choosing the appropriate experimental system—in particular, 2-dimensional versus 3-dimensional settings—to dissect the physiologic role of signaling molecules orchestrating cellular motility.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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THROMBOSIS & HEMOSTASIS

Comment on Mosnier et al, page 5970

Know your APC

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The mechanism underscoring efficacy of APC in the treatment of sepsis is still unresolved.[1] The dual nature of APC as a potent antithrombotic and cytoprotective agent complicates the task, but in this issue of *Blood*, Mosnier and colleagues offer a compelling solution and challenge the molecular underpinnings of APC function.

Ala scanning mutagenesis of the activation peptide singles out E149A, a mutant activated protein C (APC) that exhibits enhanced anticoagulant activity but greatly diminished cytoprotective effects compared with wild-type. Notwithstanding its enhanced antithrombotic activity in vivo, the variant APC is poorly effective in reducing endotoxin-induced murine mortality. Together with recent findings on a different APC variant with greatly reduced anticoagulant activity but normal cytoprotective function,[2] this important observation by Mosnier et al demonstrates that the antithrombotic activity of APC is neither necessary nor sufficient to ameliorate the outcome of sepsis.[3] It is the cytoprotective function of APC that is likely responsible for clinical efficacy, and the antithrombotic activity would only promote unwanted bleeding.

The groundbreaking discovery of the signaling properties of APC mediated by endothelial protein C receptor (EPCR)–assisted cleavage of PAR1[4] has revealed that APC is endowed with 2 distinct and physiologically important functions. APC acts as an anticoagulant by inactivating clotting factor Va with the assistance of the cofactor protein S. On the other hand, APC acts as a cytoprotective agent when it cleaves PAR1 on the surface of endothelial cells with the assistance of EPCR. Spatial separation of the underlying epitopes affords dissociation of the 2 functions by protein engineering, as previously documented in thrombin.[5] The goal is more than academic. APC variants with exclusive anticoagulant or cytoprotective activity not only provide essential reagents to dissect the functions of the enzyme in vivo, but also offer ways to improve on existing pharmacological intervention. Bleeding complications encountered in the clinical use of APC (Xigris) for the treatment of sepsis[1] could be eliminated by a variant APC that has selectively lost its anticoagulant activity.

Protein engineering of APC has already achieved important milestones. Mosnier et al have previously constructed a variant APC with greatly reduced anticoagulant activity but normal cytoprotective function.[6] The variant is as effective as wild type in reducing mortality after LPS challenge and enhances the survival of mice subjected to polymicrobial peritoneal sepsis.[3] Yang et al have recently identified a variant APC with greatly compromised cytoprotective function but normal anticoagulant activity.[7] The E149A mutant now reported by Mosnier et al further improves on the anticoagulant activity of APC in the presence of protein S at the expense of its cytoprotective function. The findings are more than a refinement of existing knowledge due to the peculiar location of E149 in the activation peptide of APC.

A patch of positively charged residues on the 30- and 70- loops (K37, K38, K39, R74 and R75) in the catalytic domain of APC provides an exosite for factor Va binding.[6] Residues E167 and E170 on the short 170-helix are important for PAR1 recognition.[7] These epitopes face the front of the enzyme and are easy targets of substrates like factor Va or PAR1 approaching the active site cleft. On the other hand, E149 is located in the back of the molecule and on the opposite side of the catalytic domain relative to the active site cleft. A fragment of the activation peptide encompassing E149 binds directly to factor Va,[8] suggesting that the epitope of this substrate extends to the back of the catalytic domain of