common integrin-independent, actin protrusion-dependent “amoeboid” migratory phenotype inside 3-dimensional environments in all hematopoietic cells. 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, 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 Coronin 1A, which results in excessive lamellipodia formation, show strongly impaired parenchymal motility. 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.

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Know your APC

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The mechanism underscoring efficacy of APC in the treatment of sepsis is still unresolved. 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.

A la 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, 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. 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 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. 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 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. 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. Yang et al have recently identified a variant APC with greatly compromised cytoprotective function but normal anticoagulant activity. 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. Residues E167 and E170 on the short 170-helix are important for PAR1 recognition. 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, suggesting that the epitope of this substrate extends to the back of the catalytic domain of
R4, but the E8A mutant is catalytically compromised, whereas E149A has normal activity toward chromogenic substrates and PAR1.

How do we reconcile the properties of the E149A mutant with our current understanding of APC signaling? The $k_{cat}/K_m$ value for the hydrolysis of PAR1 by APC under physiologic conditions is 0.0014 $\mu$M$^{-1}$s$^{-1}$, or more than 10 000-fold lower than that of the thrombin-PAR1 interaction. How can such an insignificant rate be relevant in vivo? Colocalization of EPCR and PAR1 on the membrane of endothelial cells has been invoked to solve the conundrum, but perhaps a paradigm shift is called for. APC may engage a different receptor to mediate some or most of its cytoprotective effects, or APC signaling may be amplified by the release of endogenous proteases, as recently suggested for the amplification of thrombin induced PAR1 signaling in human platelets. E149 and its neighbor residues may be involved in the recognition of other players besides EPCR and PAR1. The new findings by Mosnier et al remind us that we still do not know our APC.

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