Neutrophil mechanotransduction: A GEF to sense fluid shear stress

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Forces deriving from blood flow shear modulate vascular adherence and transendothelial migration of leukocytes into inflamed tissues, but the mechanisms by which shear is sensed are unclear. In this issue, Fine et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201603109) identify the guanosine nucleotide exchange factor GEF-H1 as critical for shear stress–induced transendothelial neutrophil migration.

Inflammation is a complex physiological process that heightens organismal defenses against intruding pathogens. In higher animals, rapid recruitment of neutrophils, a specific type of white blood cell with antimicrobial activities, is one of its earliest key events. Neutrophils produce cytotoxic chemicals, such as reactive oxygen or nitrogen species, to kill pathogens, but these chemicals also harm the host if generated in excessive amounts. Hence, neutrophil recruitment must be tightly regulated on two levels: adhesion to the endothelium and transmigration of circulating neutrophils, as well as their subsequent chemotaxis through the interstitial space to the infection locus (Kolaczkowska and Kubes, 2013).

The mechanisms that control these steps are a long-standing interest of biomedical research and often involve the molecular interactions of neutrophil surface receptors with bacterial ligands or with paracrine signals secreted by the host upon pathogen exposure. Ligand-induced structural changes in receptor molecules then activate intracellular signaling cascades that control neutrophil adhesion and migration, at least in part, through local regulation of actin polymerization and actin-myosin contraction. Small Rho family GTPases, such as Rho and Rac, are well-established key mediators of ligand-induced cytoskeletal changes in motile cells (Raftopoulou and Hall, 2004).

Inflammatory signal transduction can be regulated by structural changes of molecules or molecular assemblies caused by physical forces instead of chemical interactions. Calcium signals, which trigger cell death and inflammatory pathways, for instance, may be mediated by stretch-sensitive ion channels. Other recent examples of inflammatory mechanotransduction include the control of neutrophil polarization by plasma membrane tension (Houk et al., 2012) or swelling-induced inflammatory lipid mediator production in damaged host cells (Enyedi et al., 2013, 2016). One prominent instance of inflammatory mechanotransduction is the fluid shear stress–dependent recruitment of circulating neutrophils (Finger et al., 1996). The molecular machinery that underlies this process remains incompletely understood. In this issue, Fine et al. elegantly delineate an in vivo role for the guanosine nucleotide exchange factor GEF-H1 in shear stress–induced transendothelial migration (TEM) of neutrophils.

GEF-H1 is a microtubule-associated guanine nucleotide exchange factor (GEF) that regulates actin-myosin–dependent uropod contractility in leukocytes by promoting GDP-to-GTP exchange on the small GTPase RhoA (Birkenfeld et al., 2008). GEF-H1 has been implicated in migration and mechanotransduction of various cell types, but its involvement in shear stress–induced TEM of neutrophils had not been investigated before. GEF-H1 can be activated by microtubule depolymerization (Birukova et al., 2010; Heck et al., 2012) and other microtubule-independent mechanisms (Guilluy et al., 2011).

Fine et al. (2016) noticed decreased neutrophil infiltration into the peritoneum of GEF-H1$^{-/-}$ mice after inducing sterile peritonitis by thioglycolate injection. A similar neutrophil recruitment defect was seen after inducing sepsis by puncture of the mouse gut. When they injected differentially labeled wild-type and GEF-H1$^{-/-}$ neutrophils into mice, the wild-type cells outcompeted the GEF-H1–deficient neutrophils at the inflammation site, suggesting a cell autonomous neutrophil recruitment defect. Although neutrophils are professional antimicrobial cells that are supposed to protect the organism against bacteria, their massive recruitment into tissues during sepsis harms the host. In line with this, Fine et al. (2016) found that inhibiting septic leukocyte infiltration by neutrophil-specific GEF-H1 deletion increased mouse survival.

Intravital imaging of intravascular leukocyte behavior after exposure to *N*-formyl-methionyl-leucyl-phenylalanine (*f*MLP), a potent neutrophil-activating peptide and chemoattractant, revealed that GEF-H1$^{-/-}$ neutrophils abnormally accumulate on vessel walls, where they mostly remain static instead of crawling around. These observations support the idea that GEF-H1 is required for TEM and crawling on activated endothelia. However, when Fine et al. (2016) triggered neutrophil activation on endothelial monolayers or ICAM-1–coated surfaces ex vivo using *f*MLP, they found no difference in adhesion and migration between GEF-H1$^{-/-}$ and wild type neutrophils. As it turns out, GEF-H1 is required for mediating neutrophil responses to fluid shear. Whereas inactive GEF-H1 predominantly localized to microtubules, fluid shear promoted accumulation of active GEF-H1 within the uropod. Interestingly, nocodazole, which depolymerizes microtubules, mimicked

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some of the morphological consequences of fluid shear stress, such as cell polarization, uropod formation, and random motility, in a GEF-H1–dependent manner. In wild-type, but not GEF-H1$^{-/-}$, neutrophils nocodazole triggered actin polymerization at the cell front and myosin light chain phosphorylation at the cell back—the latter indicating increased uropod contractility. In contrast, myosin light chain phosphorylation in response to *f*MLP, CXCL1, and C5a was similar between wild-type and knockout neutrophils. Hence, neutrophil responses to paracrine signals on the one hand, and mechanical stimulation by shear on the other, appear to be transduced through different pathways.

How does the GEF-H1 pathway sense shear stress? Given the nocodazole results of this study and previous papers (Birukova et al., 2010; Heck et al., 2012), one possibility is that shear stress–induced turnover of microtubule networks releases active GEF-H1. However, microtubule stabilization by Taxol did not block shear stress–induced GEF-H1 redistribution to uropods, arguing against such a simple desequestration model. Previous studies have proposed a role for the formyl peptide receptor in fluid shear stress–sensing by neutrophils (Makino et al., 2006). Yet, the present findings indicate that *f*MLP-mediated neutrophil responses are not transduced through GEF-H1. Another recent study suggested that fluid shear augments leukocyte activation by platelet activating factor presented to neutrophils on the surface of endothelial cells (Mitchell et al., 2014). However, shear-induced migration was also observed on ICAM-1–coated surfaces, which suggests that leukocyte surface adhesion suffices for shear stress transduction, maybe through formation of mechanosensitive "catch bonds" (Kong et al., 2009; Sundd et al., 2013).

Aside from the precise mechanosensing mechanism, it will be interesting to determine the physiological benefits of shear stress detection by neutrophils. Notably, the present study shows that septic mice survive better if their neutrophils cannot sense fluid shear. Under which conditions does fluid shear-sensing provide an advantage for the host that could explain why such a mechanism developed in the first place? Neutrophil-specific deletion of GEF-H1, as reported here by Fine et al. (2016), could be a powerful, new tool to address this question.

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