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blood commentary

PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Salvermoser et al, page 1887

Myo1f is critical for neutrophil migration in vivo

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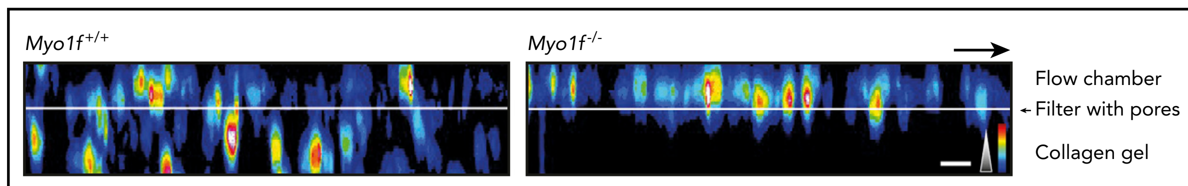
In this issue of *Blood*, Salvermoser et al demonstrate that the unconventional class I myosin 1f (Myo1f) is crucial for neutrophil trafficking during acute inflammation in vascular beds in vivo. Importantly, they demonstrate that Myo1f is crucial for the dynamics of the deformation of the neutrophil nucleus and consequent capacity of these cells to extravasate in vivo and to negotiate through three-dimensional (3D) environments.[1]

Neutrophils are polymorphonuclear leukocytes known to play a crucial role in acute infection. Indeed, in neutropenic individuals or in conditions in which neutrophil migration to tissue is deficient, there is significant risk of death because of bacterial and fungal infections. To deal with invading microorganisms, neutrophils circulating in blood need to interact with endothelial cells, transmigrate into the relevant tissue, and negotiate their way into the site of infection. Much is known about the processes and molecular interactions needed for neutrophil recruitment and accumulation in tissues, which have been reviewed elsewhere.[2] Significant knowledge in the field has been gained by using in vitro models that try to mimic the complex process of neutrophil extravasation in vivo. In this regard, static and usual chemotaxis assays have been instrumental in the characterization of chemoattractant molecules, adhesion molecules, and intracellular pathways necessary for neutrophil recruitment.

Salvermoser and colleagues show that in mice deficient for the protein Myo1f (Myo1f$^{-/-}$ mice), neutrophil migration after induction of acute inflammation is decreased in multiple vascular beds, including the cremaster muscle, the peritoneal cavity, and the lungs. This study confirms findings of Kim and colleagues,[3] who showed decreased neutrophil recruitment and defects in host defense in Myo1f$^{-/-}$ mice. However, the study by Salvermoser et al advances the field significantly by providing detailed information on the phenotype and suggesting a potential mechanism for the defect. The authors show in the paper and accompanying illustrations that Myo1f was not needed for spreading, polarization, and mechanotactic migration under flow conditions as well as chemotactic migration in two-dimensional (2D) environments. The latter results are in agreement with in vivo findings showing that leukocyte rolling flux fraction, rolling velocity, and the number of adherent leukocytes in TNF-stimulated cremaster muscle venules was similar in wild-type and Myo1f$^{-/-}$ mice. Therefore, the observed effects on extravasation were likely not because of altered interactions with endothelial cells in vitro under conditions of flow or in vivo. This is in contrast with enhanced adhesiveness to adhesion molecules under static conditions. Two major messages derive from these findings: (1) increased adhesiveness observed under static conditions was not responsible for the extravasation defect; and (2) static assays, although useful to study certain characteristics of neutrophil behavior, may fail to mimic the rich interactions that occur in vivo.

Subsequent experiments showed that, in contrast to the lack of observed effects in the 2D environment, Myo1f was crucial for neutrophil transmigration through physical barriers and in a 3D environment. Significantly, the authors show unequivocally that Myo1f was required for migration in 3D environments to enable the squeezing of the nucleus through narrow spaces (see figure). Migration of human neutrophils in a restrictive barrier of a meshwork of collagen fibers is initiated by the formation of a small nuclear lobe



Under physiological flow conditions (1 dyne/cm^2), murine neutrophils attach to membranes coated with recombinant murine ICAM-1 (rmICAM-1) and rmP-selectin and successfully transmigrate through pores into collagen gel containing a chemoattractant molecule. In contrast, Myo1f-deficient neutrophils are mostly unable to transmigrate, and the majority of neutrophils are found within the flow chamber or stuck within the pores. This figure provides a robust demonstration of the need for Myo1f for neutrophils to migrate through 3D environments in vitro and an explanation for the failure of Myo1f-deficient neutrophils to accumulate into sites of inflammation in vivo. See the complete Figure 3 in the article by Salvermoser et al that begins on page 1887.

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preparing the deformation of the nucleus. This step is followed by further deformation and elongation of the nucleus and, once migration is completed, the nucleus is refolded into a roundish, multilobular shape.[4] Nuclear deformation has been shown to be required for neutrophil migration within tissues with pore cross sections of 2 to 20 μm.[5] This process was significantly impaired in Myo1f-deficient murine neutrophils or HL-60 cells stably expressing EGFP-Myo1f. Altogether, these studies suggest that defective nucleus deformation was the reason for impaired 3D migration and accumulation in vivo in the genetic absence of Myo1f.

Class I myosins are widely expressed members of the myosin superfamily that bind to actin filaments and hydrolyze adenosine triphosphate to produce mechanical force.[6] These proteins bind to membranes by their basic tail homology 1 (TH-1) domains and may be important for membrane-associated functions, such as endocytosis, cell signaling, and cell motility. Myo1f was found to be mainly expressed in neutrophils.[3] Although the neutrophil nucleus may be quite malleable, the nucleus-cytoskeleton connection is essential for 3D migration by transmitting force from the cytoskeleton to the inside of the nucleus.[7] An interesting possibility raised from the studies of Salvermoser and colleagues is that Myo1f may link the cytoskeleton to the nuclear envelope via its TH1 domain to provide a high malleability of the neutrophil nucleus. This possibility clearly deserves further investigation.

Lipopolysaccharide-induced neutrophil extravasation into the lung interstitium and in the bronchoalveolar space was significantly impaired in Myo1f$^{-/-}$ mice as compared with Myo1f$^{+/+}$ mice. In contrast, there was increased neutrophil accumulation within capillaries of the lungs of Myo1f$^{-/-}$ mice. Interestingly, it has been shown that various mediators of inflammation can cause neutrophil sequestration in the lungs and other capillaries during acute inflammation by increasing the stiffness of the neutrophils. Stimulated neutrophils (diameter, 8 μm) are retained in pulmonary capillaries (5.5 μm) as a result of a decreased ability of the cell to deform within the capillary in response to the hydrodynamic forces of the bloodstream.[8] Increased neutrophil stiffness and retention in the lungs are thought to contribute to their migration into the lung parenchyma and airway spaces.[9] It will be very interesting to evaluate the relevance of Myo1f in this context, as activation and retention of neutrophils within capillaries may contribute to lung injury and the systemic inflammatory response syndrome secondary to sepsis. Clearly, Myo1f may contribute not only to the beneficial role of neutrophils to fight infection, but also to the role these cells play in acute and chronic inflammatory diseases not caused by infection.

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

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CLINICAL TRIALS AND OBSERVATIONS

Comment on O'Brien et al, page 1910

Ibrutinib: coming of age?

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In this issue of *Blood*, O'Brien et al report long-term efficacy and safety of ibrutinib in the first cohort of patients with chronic lymphocytic leukemia (CLL) treated, now with 5-year follow-up, the longest of any CLL cohort to date.[1]

Ibrutinib has come into widespread use since its initial US Food and Drug Administration approval for relapsed CLL 4 years ago, based on the early results of this same study.[2] Although many other studies have confirmed the high efficacy of ibrutinib in CLL, follow-up has remained quite short. Thus, many questions about durability and predictors of response, as well as long-term tolerability, have remained unanswered. In this study of high-risk patients with relapsed/refractory disease, the median progression-free survival (PFS) was reached at a strikingly good 51 months, which compares favorably to that achieved with older regimens in a comparable patient population.[3] The median treatment duration for the relapsed/refractory cohort was 39 months, with 33% discontinuing for disease progression. Interestingly, the PFS curves by cytogenetic abnormality have a distribution similar to that of the classic survival curves of Döhner et al[4] (see figure panel A), with del(17p) remaining highest risk, with a median PFS of 26 months. Complex karyotype has emerged as a predictor of shortened PFS in ibrutinib studies,[5] and in this study, it was associated with a 31-month PFS, driven significantly by co-occurrence with del(17p) (see figure panel B). The extent to which the adverse prognosis of complex karyotype is driven by its association with del(17p) remains unknown, but clearly, this needs to be investigated prospectively in future ibrutinib studies.

Overall, these findings seem aligned with the recently reported 59% 3-year PFS achieved with ibrutinib in the confirmatory RESONATE trial,[6] which randomly assigned patients with relapsed CLL to ibrutinib or ofatumumab. In the study by O'Brien et al,