Activating Integrins Isn't Always "Beta" for Neutrophil Migration!

The lungs possess extraordinary defenses against bacteria and other stimuli. Initial mechanisms of host defense include the very effective mucociliary escalator and the alveolar macrophages, which clear many stimuli without inducing an inflammatory response and recruitment of circulating leukocytes. When these defenses are overwhelmed, epithelial cells and macrophages call in help from outside the lungs. In response to bacteria, neutrophils are usually first to arrive, and their response is quick, recognizing a need within minutes.

Neutrophil adhesion and migration within the pulmonary microvasculature are unique in important ways. Neutrophil emigration into the distal lung occurs primarily through the capillary bed, rather than the postcapillary venules, where the paradigm of leukocyte rolling followed by firm adhesion and migration was developed (1, 2). The pulmonary capillaries have a complex geometry of branching segments, and the diameter of at least half the segments is narrower than a spherical neutrophil so that rolling cannot occur (3–6). Although the leukocyte adhesion complex CD11/CD18 is not always required for neutrophil emigration in the lungs, *Pseudomonas aeruginosa* induces CD11/CD18-dependent neutrophil recruitment (6–8).

CD18 is the β_2-integrin subunit mediating both outside-in signaling after binding of a ligand and inside-out signaling that results in activation of the adhesion complex through a two-step process (9–11). Clustering of integrins induced by ligand binding can initiate association of two cytosolic proteins, talin-1 and kindlin-3, with two distinct sites on the short cytoplasmic tail of CD18. Considerable data suggest that talin-1 initiates the conformational change in all β-integrins from a compact/bent state to an extended closed headpiece (intermediate binding affinity), whereas kindlin-3 is required for transformation of this intermediate state to an extended open headpiece state with a high binding affinity for ligands (9, 10).

In this issue of the *Journal*, Wilson and colleagues (pp. 620–627) elegantly ask critical questions about the requirements for talin-1 and kindlin-3 in neutrophil migration during *P. aeruginosa*–induced pneumonia (12). In striking contrast to studies showing that these molecules are required for neutrophil transmigration into the peritoneum (11), talin-1 is not required for neutrophil recruitment into the lungs. Moreover, kindlin-3–null neutrophils migrate in greater numbers than wild-type neutrophils, suggesting that kindlin-3–null neutrophils have an advantage. Importantly, antibody blockade shows that CD18 is still required for emigration of kindlin-3–null neutrophils.

Studies to determine the site within the lungs where neutrophils enter more abundantly to account for this advantage show that the neutrophils lacking either talin-1 or kindlin-3 have no defect in sequestering within the lung capillaries, but do have an advantage in migrating into the interstitium compared with wild-type neutrophils, suggesting that activated integrins may slow neutrophil transit through the interstitium (12). The mutant neutrophils' advantage does not further increase as they migrate from the interstitium into the lavageable airspace. Walker and colleagues (2, 13, 14) suggest that the path neutrophils take is through the endothelial junctions on the thick side of the capillaries, fully accumulating within the interstitium, and then migrating between type I and type II alveolar epithelial cells into the alveolar space. Taken together, activated integrins appear to slow neutrophil migration through the interstitium, but have less impact on migration through the alveolar epithelium. An alternative explanation, that activated integrins slow neutrophil migration through the epithelium and result in a "backup" in the interstitium, seems unlikely.

These studies are elegantly performed using mice, the bone marrow of which has been reconstituted with a mixture of mutant and wild-type bone marrow, allowing comparison of each genotype within the same mouse. The radiation dose (10 Gy) does induce a lung injury, including replacement of alveolar macrophages with bone marrow–derived cells and an immune cell infiltrate within the bronchovascular bundle, but an impact of this on the results seems unlikely. These studies have the technical caveats that flushing the vasculature does not completely differentiate between the circulating and sequestering neutrophils, and that bronchoalveolar lavage does not remove all the neutrophils that have emigrated into the alveoli. One does wonder whether neutrophils that cannot transform their integrins into a high activation state might be less sticky and more lavageable than wild-type neutrophils, but the major process that activated integrins appear to be impeding is trafficking through the interstitium.

Furthermore, these studies address the effect of a β_2-integrin allosteric antagonist, XVA143, that mimics the deficiency of kindlin-3 by preventing the transformation from mid to high affinity (12). Importantly, XVA143 also results in greater numbers of neutrophils in the interstitium and the bronchoalveolar lavage fluid. This two- to threefold increase in neutrophils does not increase lung injury, as measured by wet-to-dry weights, or alter bacterial clearance. Studies using immortalized myeloid progenitors differentiated toward a neutrophil phenotype show that generation of reactive oxygen species in response to *P. aeruginosa* is not altered by deficiency of either talin-1 or kindlin-3, but phagocytosis is reduced by about half. Why this defect in phagocytosis does not result in less clearance of bacteria from the lungs is not clear, but perhaps sufficient numbers of neutrophils are recruited to overcome this defect. Whether XVA143 has therapeutic use as an enhancer of neutrophil recruitment, perhaps during antibiotic-resistant infections, remains to be determined.

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As with all forefront work, these studies generate new questions. Particularly important seem questions about the mechanisms through which activated β_2- (or β_1-) integrins are slowing neutrophil migration compared with unactivated integrins. Does this slower transit act to facilitate the sealing of the endothelium during migration of neutrophils between endothelial junctions and to prevent leakiness? Alternatively, does the braking effect of activated integrins enhance endothelial or interstitial injury during diapedesis? Careful measurements of permeability and insight into the differences between activated and nonactivated integrins will be exciting to pursue. Another way to think about these next questions may be to ask, how does activation of integrins result in less neutrophil migration? Does the greater phagocytosis seen in neutrophils with activated integrins result in faster clearance of the stimulus, and thus more rapid turnoff of neutrophil migration? Or do neutrophils with activated integrins produce an inhibitor of neutrophil recruitment (or less neutrophil chemokines)? And do activated integrins impact on resolution of pneumonia, when assessed at later stages? Studies examining neutrophils lacking *Skap2*, which is required for binding of both talin-1 and kindlin-3 to β_2-integrins and their activation (15), might also be interesting. The innovative and ingenious studies reported by these investigators (12) hugely enhance our knowledge about neutrophil recruitment in lung disease and show that activating integrins isn't always "beta" for neutrophil migration! ■

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References

1. Downey GP, Worthen GS, Henson PM, Hyde DM. Neutrophil sequestration and migration in localized pulmonary inflammation: capillary localization and migration across the interalveolar septum. *Am Rev Respir Dis* 1993;147:168–176.
2. Burns AR, Smith CW, Walker DC. Unique structural features that influence neutrophil emigration into the lung. *Physiol Rev* 2003;83: 309–336.
3. Wiggs BR, English D, Quinlan WM, Doyle NA, Hogg JC, Doerschuk CM. Contributions of capillary pathway size and neutrophil deformability to neutrophil transit through rabbit lungs. *J Appl Physiol (1985)* 1994;77: 463–470.
4. Doerschuk CM, Beyers N, Coxson HO, Wiggs B, Hogg JC. Comparison of neutrophil and capillary diameters and their relation to neutrophil sequestration in the lung. *J Appl Physiol (1985)* 1993; 74:3040–3045.
5. Gebb SA, Graham JA, Hanger CC, Godbey PS, Capen RL, Doerschuk CM, Wagner WW Jr. Sites of leukocyte sequestration in the pulmonary microcirculation. *J Appl Physiol (1985)* 1995;79:493–497.
6. Doerschuk CM. Mechanisms of leukocyte sequestration in inflamed lungs. *Microcirculation* 2001;8:71–88.
7. Doerschuk CM, Winn RK, Coxson HO, Harlan JM. CD18-dependent and -independent mechanisms of neutrophil emigration in the pulmonary and systemic microcirculation of rabbits. *J Immunol* 1990;144:2327–2333.
8. Mizgerd JP, Horwitz BH, Quillen HC, Scott ML, Doerschuk CM. Effects of CD18 deficiency on the emigration of murine neutrophils during pneumonia. *J Immunol* 1999;163:995–999.
9. Stadtmann A, Zarbock A. The role of kindlin in neutrophil recruitment to inflammatory sites. *Curr Opin Hematol* 2017;24:38–45.
10. Calderwood DA, Campbell ID, Critchley DR. Talins and kindlins: partners in integrin-mediated adhesion. *Nat Rev Mol Cell Biol* 2013; 14:503–517.
11. Lefort CT, Rossaint J, Moser M, Petrich BG, Zarbock A, Monkley SJ, Critchley DR, Ginsberg MH, Fässler R, Ley K. Distinct roles for talin-1 and kindlin-3 in LFA-1 extension and affinity regulation. *Blood* 2012; 119:4275–4282.
12. Wilson ZS, Ahn LB, Serratelli WS, Belley MD, Lomas-Neira J, Sen M, Lefort CT. Activated β_2 integrins restrict neutrophil recruitment during murine acute pseudomonal pneumonia. *Am J Respir Cell Mol Biol* 2017;56:620–627.
13. Behzad AR, Chu F, Walker DC. Fibroblasts are in a position to provide directional information to migrating neutrophils during pneumonia in rabbit lungs. *Microvasc Res* 1996;51:303–316.
14. Walker DC, Behzad AR, Chu F. Neutrophil migration through preexisting holes in the basal laminae of alveolar capillaries and epithelium during streptococcal pneumonia. *Microvasc Res* 1995; 50:397–416.
15. Boras M, Volmering S, Bokemeyer A, Rossaint J, Block H, Bardel B, Van Marck V, Heitplatz B, Kliche S, Reinhold A, *et al*. Skap2 is required for β2 integrin–mediated neutrophil recruitment and functions. *J Exp Med* 2017;214:851–874.