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## Vinculin in Neutrophil Adhesion, Motility and Trafficking

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Neutrophils are innate immune effector cells that migrate from the blood to resolve bacterial and fungal infections. Understanding how neutrophils migrate is critical for regulating excessive inflammation and subsequent collateral injury.  $\beta 2$  integrins are essential to classical neutrophil recruitment from the blood, and the activation of  $\beta 2$  integrins has been well defined in previous studies. Adhesion stabilization of neutrophils on the endothelial surface as they crawl into a favorable position for transmigration is not as well defined. Neutrophils do not make mature focal adhesions, but do express the focal adhesion protein vinculin. Vinculin associates with integrins by binding to talin-1 and stabilizes integrin adhesions by recruiting various actin-associated proteins or by associating with actin directly. This study characterizes the role of vinculin in neutrophil  $\beta 2$  integrin-dependent adhesion, motility and anti-bacterial function. Intrinsic activation of  $\beta 2$  integrins is unaffected by vinculin knockout after CXCL1 activation. Vinculin knockout attenuates neutrophil adhesion, spreading, and motility on glass coated with  $\beta 2$  integrin ligand, ICAM-1, and activating CXCL1. Vinculin knockout also reduces neutrophil spreading in response to ICAM-1/CXCL1 on polyacrylamide gels of high stiffness but not lower stiffness. Vinculin knockout reduces traction stresses of neutrophils and the actin stiffening response after stimulation. Unlike static conditions, vinculin knockout does not affect neutrophil motility under flow conditions. Vinculin knockout attenuates respiratory burst, but does not affect phagocytosis. In mixed chimeric mice given intraperitoneal thioglycollate, we find comparable migration of vinculin-knockout and vinculin-sufficient neutrophils into the peritoneum. Altogether, while vinculin enhances neutrophil  $\beta 2$  integrin adhesion strength, vinculin knockout does not affect neutrophil motility and trafficking under physiological conditions.

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