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Immunity

The Neutrophil Btk Signalosome Regulates Integrin Activation during Sterile Inflammation

Highlights

- Btk is required for neutrophil recruitment during sterile inflammation
- Btk is required for selective fMLF-triggered Mac-1, but not LFA-1, activation
- Hck, WASp, and PLCγ2 are key signaling molecules in the fMLF-triggered pathway
- Btk is required for integrin-mediated outside-in signaling and FcRγ-mediated functions

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In Brief

The β_2-integrin Mac-1 is crucial for neutrophil recruitment during sterile inflammation. Zarbock and colleagues demonstrate that the Btk signalosome—consisting of Hck, Btk, WASp, and Plcγ2—is indispensible for Mac-1-dependent functions.

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The Neutrophil Btk Signalosome Regulates Integrin Activation during Sterile Inflammation

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SUMMARY

Neutrophils are recruited from the blood to sites of sterile inflammation, where they are involved in wound healing but can also cause tissue damage. During sterile inflammation, necrotic cells release pro-inflammatory molecules including formylated peptides. However, the signaling pathway triggered by formylated peptides to integrin activation and leukocyte recruitment is unknown. By using spinning-disk confocal intravital microscopy, we examined the molecular mechanisms of leukocyte recruitment to sites of focal hepatic necrosis in vivo. We demonstrated that the Bruton's tyrosine kinase (Btk) was required for multiple Mac-1 activation events involved in neutrophil recruitment and functions during sterile inflammation triggered by fMLF. The Src family kinase Hck, Wiskott-Aldrich-syndrome protein, and phospholipase Cγ2 were also involved in this pathway required for fMLF-triggered Mac-1 activation and neutrophil recruitment. Thus, we have identified a neutrophil Btk signalosome that is involved in a signaling pathway triggered by formylated peptides leading to the selective activation of Mac-1 and neutrophil recruitment during sterile inflammation.

INTRODUCTION

Neutrophils are key players in acute inflammation. They play an important role in host defense and contribute to inflammation-related tissue damage. Necrotic cell death can induce sterile inflammation characterized by the recruitment of innate immune effector cells into the damaged tissue. The recruited neutrophils contribute to the clearance of debris, but they can also cause profound collateral tissue destruction due to the release of their vast arsenal of hydrolytic, oxidative, and pore-forming molecules (McDonald and Kubes 2012). Excessive neutrophil recruitment during sterile inflammation accounts for the immunopathology observed in many diseases, including trauma, autoimmunity, ischemic injuries, and sterile liver injury (Imaeda et al., 2009; McDonald et al., 2010). Therefore, understanding the mechanisms for neutrophil recruitment is of major physiological and pathophysiological importance.

Several endogenous pro-inflammatory damage-associated molecular patterns (DAMPs), including lipid mediators, N-formylated peptides, and extracellular matrix proteins, are released during cell death by necrosis (McDonald and Kubes 2012; McDonald et al., 2010; Imaeda et al., 2009). Neutrophils express a variety of receptors that recognize N-formylated peptides, including those specific for the prototype ligand formyl-methionyl-leucyl-phenylalanine (fMLF). Eliminating one of the receptors for fMLF (*Fpr1*$^{-/-}$) results in a reduced neutrophil recruitment into the inflamed lung (Grommes et al., 2014) and reduces neutrophil adhesion in the liver during sterile inflammation (McDonald et al., 2010), highlighting the importance of cell stimulation with N-formylated peptides in innate immunity.

Receptors for fMLF are $G\alpha_i$-linked receptors that trigger a variety of intracellular signaling pathways (Dorward et al., 2015), provoking different cell responses like neutrophil chemotaxis, respiratory burst, and transcriptional regulation. Activation of phosphoinositide 3-kinase γ (PI3Kγ) and phospholipase C (PLC) isoforms are the predominant signaling events upon fMLF-receptor activation. PI3Kγ induces the conversion of phosphoinositol-4,5-biphosphate to phosphoinositol-3,4,5-triphosphate which is involved in neutrophil cytoskeletal reorganization and chemotaxis. The phospholipase Cβ (PLCβ) isoform is required for the production of diacylglycerol (DAG) and inositol-3,4,5-trisphosphate (IP_3), which induces release of intracellular calcium into the cytoplasm (Dorward et al., 2015).

In addition to the activation of PI3K and PLC, fMLF receptors trigger a rapid tyrosine phosphorylation of several signaling molecules in neutrophils, including Src family kinases (SFKs) and Tec family kinases (Zarbock and Ley, 2011; Gilbert et al., 2003; Futosi et al., 2013). The SFKs Fgr, Hck, and Lyn are expressed in neutrophils and are involved in several signaling pathways by promoting phosphorylation of downstream effectors (Thomas and Brugge, 1997; Lowell and Berton, 1999). These SFKs share a high degree of structural homology and possess three major domains: a Src homology 3 (SH3) domain, a SH2 domain, and the tyrosine kinase (SH1) domain (Thomas and Brugge, 1997). SFKs can be activated by several molecules and participate in a variety of cell functions in neutrophils (Zarbock and Ley, 2011; Thomas and Brugge, 1997; Lowell and Berton, 1999). They also modulate