ABSTRACT# 48

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Title: Ezrin is a key regulator of lung interstitial macrophage response to LPS
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Introduction: Lung tissues are populated by resident macrophages (interstitial (IMs) and alveolar (AMs)), and, in response to infections, by recruited circulating monocytes (moMs). Cystic fibrosis (CF) lung disease is associated with dysregulated MΦ function. We have previously shown that level of ezrin, an actin binding protein important for CFTR stabilization at the plasma membrane (PM), is reduced in murine and human CF MΦ when compared to non-CF cells. Little is known about the ezrin' function in macrophages during lung immune response. Our overall hypothesis is that ezrin, by controlling cortical actin organization, fine-tunes activation of lung macrophages in response to infections.

Methods: We generated a mouse model in which ezrin is specifically knocked out in monocyte/MΦ (Ezrinfl/fl x Cx3Cr1Cre/+; hereafter Ez-KO/Cx3cr1 ). Control (Cx3Cr1Cre/+ ) and Ez-KO/Cx3cr1 mice (n=3 per genotype) were nebulized with LPS from P. aeruginosa (12.5 mg/dose) over 15 minutes. Flow cytometry was used to characterize monocyte/MΦ populations in lung tissues. FACS was used to collect lung MΦ populations for expression analysis (RT-qPCR).

Results: In untreated control mice, ezrin is equally expressed in all lung MΦ populations, while, in response to LPS, IMs show 4-fold increased level compared to moMs and AMs. Consistently, Ez-KO/Cx3cr1 mice have a statistically significant reduction of IM number compared to Cx3Cr1Cre/+ in response to LPS. Lack of ezrin does not affect number of moMs and AMs. Peripheral blood monocytes were also not different among genotypes. Compared to Cx3Cr1Cre/+ , Ez-KO/Cx3cr1 IMs are more pro-inflammatory, as shown by higher expression of Il6, Tnfa, and Cxcl1. In vitro studies show that in response to LPS, Ez-KO/Cx3Cr1 MΦs have stunted filopodia at the PM, altered F-actin distribution and reduced cell volume. Moreover, expression of integrins Itgb1 and Itgα1 is highly impaired in absence of Ezrin.

Conclusions Our results show that ezrin plays a critical role in the IMs response to the inflamed lung microenvironment, affecting their number and activation state. Decreased expression of integrins, altered actin organization and cellular shape observed in absence of ezrin suggest that this protein may mediate the adhesion of IMs to the lung extracellular matrix.

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