Loss of caveolin-1 expression leads to decreased calcium flux in freshly isolated airway smooth muscle cells

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Rationale: Caveolin-1 (cav-1) is the marker protein of caveolae, which are a specialized subset of lipid rafts. Cav-1 is highly expressed within the lung in epithelial cells, endothelial cells, various immune cells, and airway smooth muscle (ASM). Caveolae are thought of as membrane platforms that can concentrate and organize signaling proteins, leading to more rapid and effective activation of the signaling cascade. Additionally, it has been shown that cav-1 directly participates in various signaling pathways. We have previously reported (Brown et al., Am. J. Respir. Crit. Care Med., Apr 2009; 179: A5093) that the caveolin-1 deficient (cav-$1^{-/-}$) mice exposed to aerosolized lipopolysaccharide (LPS) have decreased airway hyperresponsiveness (AHR) to methacholine challenge as compared to wild-type (WT) mice. We also showed that this decrease does not directly correlate with the inflammatory response, as inflammatory cytokine and chemokine levels were increased in the bronchoalveolar lavage fluid of cav-$1^{-/-}$ mice compared to WT mice. The reduced AHR was instead attributed to decreased contraction of ASM observed in an ex vivo challenge of isolated bronchial rings. In order to investigate the mechanism behind this decreased contraction, we measured cholinergic agonist-induced calcium responses in freshly isolated ASM cells and muscarinic receptor expression in lungs from cav-$1^{-/-}$ mice and their littermate WT controls.

Methods: Intact tracheas and lungs were removed from untreated mice. The tracheas were cleaned of surrounding tissue, epithelial cells were brushed off, and the remaining tissue was minced and digested. The resulting cell suspension was plated and cells were allowed to adhere in culture. The lungs were homogenized and analyzed by Western blot.

Results: Cav-$1^{-/-}$ ASM had a 60% decrease in carbachol induced calcium responses when compared to WT ASM. The expression level of the carbachol receptor, muscarinic receptor 3 (M3), was similar in whole lung homogenates from WT and cav-$1^{-/-}$ mice.

Conclusions: Loss of cav-1 expression in ASM results in decreased calcium flux which is not a result of reduced M3 receptor expression. Taken together, our combined data suggest that cav-1 plays an integral role in airway responsiveness, and that its mechanism of action lies downstream of the M3 receptor in ASM. In addition, cav-1 polymorphisms, which have been reported in human studies, may have the potential to influence the susceptibility to inflammatory lung diseases and the severity of AHR. Funded in part by HL-30923, HL-68071, and HL-84917.