Rhinovirus C Infection Activates Cytoskeletal Signaling and Results in Barrier Loss in Highly Differentiated Airway Epithelial Cells

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RATIONALE: Human rhinovirus (HRV)-induced wheezing illnesses are a major risk factor for the development of childhood asthma with mounting evidence that clinical outcome may be strain-specific. HRV-C group members are associated with more severe exacerbations in asthmatic children and increased hospitalizations. Although the replication cycles of HRV-A and B strains have been extensively studied using cells of non-airway origin (e.g. HeLa cells), HRV-C exclusively infects highly differentiated human airway epithelial cells (HAE) and little is known about its pathogenic nature. It has been reported that asthmatics have an altered epithelial phenotype, which is characterized by increased epithelial fragility and altered barrier function. We sought to determine the role of HRV-C infections in driving this altered epithelial phenotype in asthmatic children; we hypothesized that: HRV-C alters cytoskeletal signaling during infection, resulting in barrier dysfunction and subsequent cell extrusion as infection progresses. We speculate that HRV-C infection of asthmatic HBE undergo enhanced cytoskeletal signaling activation, leading to enhanced barrier loss and worse clinical outcome. METHODS: Highly differentiated air-liquid interface (ALI) cultures of HBE, isolated from normal human lungs, were infected with HRV-C15 for 4, 8, 12, 16, 20, 24 hours. HRV-C15 replication was evaluated using cell lysates to quantify intracellular viral RNA by RT-PCR. Matched barrier function studies measuring apical to basolateral FITC-dextran (3-5 kDa) flux were performed at each time point. Cytoskeletal remodeling studies were performed using the following inhibitors: Y-27632 (Rho-associated protein kinase), ML-7 and ML-9 (myosin light chain kinase (MLCK)); and evaluated by Western blot. Immunofluorescence staining of ZO-1 was performed to evaluate cytoskeletal remodeling using a resonant scanning confocal microscope. RESULTS: HRV-C15 causes remodeling of epithelial architecture during peak infection at 24 hours, which is marked by increased phosphorylation of myosin light chain (MLC) II, and high apical to basolateral FITC-dextran flux. Inhibiting ROCK significantly improves barrier function and reverses HRV-C15 driven phosphorylation of MLC, while inhibiting MLCK does not restore barrier. HRV-C15 infection does not affect the overall expression of Rho-associated signaling proteins (i.e., RhoA, LIMK, ROCK). By contrast, HRV-C15 activates upstream Rho-GTPase activity before barrier loss occurs, indicating a critical role for early cytoskeletal signaling in HRV-C15 induced barrier loss and subsequent cell extrusion. CONCLUSION: We conclude that HRV-C serotype dramatically remolds cellular architecture in HAE that is associated with enhanced Rho-GTPase signaling and loss of barrier function. Future studies will evaluate the role of these cytoskeletal signaling pathways on cell extrusion and post-infection recovery.

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