Relationship Between Ace2 and Tmprr2 Expression by Differentiated Primary Bronchial Airway Epithelial Cells and SarsCov2 Replication

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Rationale: SARS-CoV-2 gains entrance to airway epithelial cells (AECs) via binding of the viral spike protein to the angiotensin-converting enzyme 2 (ACE2) on the cell surface, and the serine protease TMPRSS2 is thought to play an important role in facilitating SARS-CoV-2 entry by priming the spike protein. There is some data suggesting that ACE-2 expression by AECs is greater in adults than children, leading many to hypothesize that airway ACE-2 expression is a risk factor for SARS-CoV-2 replication and COVID-19 disease. Aim: Determine whether expression of ACE-2 and/or TMPRSS2 by bronchial AECs from children and adults is associated with SARS-CoV-2 replication. Methods: Primary bronchial AECs from children and adults (n=18; ages 8-75 yrs.) were differentiated ex vivo at an air-liquid interface to generate organotypic cultures. In a biosafety level 3 (BSL-3) facility, cultures were infected with SARS-CoV-2 isolate USA-WA1/2020 at a multiplicity of infection (MOI) of 0.5. At 96 hrs. following infection, RNA and protein were isolated from cultures. SARS-CoV-2 replication in cultures was assessed by PCR, and quantified as viral copy number/ng RNA. ACE-2 expression was assessed by qPCR in both SARS-CoV-2 infected AEC cultures and uninfected control cultures. In a subset of subjects (n=6), ACE-2 expression was measured in paired nasal and bronchial AEC cultures. Finally, we assessed the effect of apical treatment of AEC cultures with recombinant ACE-2 (rACE-2) prior to SARS-CoV-2 and once daily for 96hrs. Results: In the primary bronchial AECs studied we observed marked between subject heterogeneity in ACE-2 expression (14-fold), TMPRSS2 expression (8-fold), and SARS-CoV-2 replication (range 167 - 89,040 copies/ng RNA). Baseline ACE-2 expression in uninfected AECs correlated with SARS-CoV-2 replication in infected AECs (Spearman r=0.6, p=0.02), whereas TMPRSS2 expression was not associated with viral replication (r=-0.2, p=0.5). In paired nasal and bronchial AEC cultures ACE-2 expression was strongly correlated (Pearson R²=0.66, p=0.05). Treatment of AECs with rACE-2 added apically immediately prior to infection and refreshed daily for 96 hrs. across a range of concentrations (0.1-1000 ng/mL rACE in 100μL of PBS; n=4 AEC primary lines) led to a marked reduction in SARS-CoV-2 replication (mean of 5040 viral copies/ng RNA in untreated AECs to 16 viral copies/ng RNA at 10ng/mL). Conclusion: Expression of ACE-2 by primary bronchial AECs from children and adults is heterogenous, and is associated with SARS-CoV-2 replication ex vivo. ACE-2 expression by AECs may partially explain the between subject variability in the risk and severity of COVID-19.

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