**Title:** Differential responses of monolayer and differentiated airway epithelial cell cultures to NTHi infection

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**Body:** The innate defence functions of the lung require a patent airway epithelium and infections are often associated with epithelial defects and phenotypic alterations. Non-typeable Haemophilus influenzae (NTHi) one of the first bacterial species to infect children, reduces innate defences, allowing further colonisation with other pathogens, including RSV. We have established NTHi infections of lung derived cell lines and primary airway cells in differentiated cultures prior to the establishment of secondary infections with RSV as a model for paediatric RSV infection. A549, H292 and primary airway epithelial cells were grown in monolayer cultures in transwell inserts. Differentiated cultures of tracheobronchial epithelial (TBE) cells were grown at the ALI using established methods. The apical compartments of established cultures were infected with increasing doses of GFP tagged NTHi and followed for up to 7 days. Infection and cell viability was determined using confocal microscopy and bacterial counts at each time point. A549 and H292 cells, and undifferentiated primary cells became heavily infected and by day 7 almost complete loss of cells was associated with a loss of viable bacteria. Cytokine array studies showed that these cultures mounted limited cytokine responses. The ALI TBE cell cultures had an enhanced ability to overcome the same bacterial infections and this was associated with a marked cytokine response. This data suggests that differentiated epithelial cell cultures have an enhanced ability to overcome bacterial infection compared to monolayer cultures of epithelial cells. This is likely due to the innate defensive shield secreted from these complex cultures.