In vitro and in vivo studies with recombinant clinical isolate-based respiratory syncytial virus in primary respiratory epithelial cultures, 2D airway organoids and small animal models

Department of Viroscience, Postgraduate School of Molecular Medicine, Erasmus MC, Rotterdam, The Netherlands. Center for Vaccine Research, University of Pittsburgh, Pittsburgh, PA, USA.

Introduction

Human respiratory syncytial virus (HRSV) usually causes mild respiratory tract infections, but a fraction of infants develops severe respiratory disease. From severe HRSV cases we learned that HRSV mainly infects ciliated epithelial cells in the airways. Subsequent inflammation and mucus production leads to plugging and occlusion of the small airways. In cell culture models, HRSV infection levels and inflammatory responses have predominantly been studied around the peak of infection. However, what happens at early time points remains largely unknown.

Our aim was to study the replication kinetics of HRSV-A and HRSV-B in vitro and to study the early viral spread of HRSV-A in vivo, because we hypothesize that this contributes to the risk of severe HRSV disease.

Methods

(1) HRSV-A replicates faster than HRSV-B in cells from both the URT and LRT.
(2) HRSV-A infection in 2D AO cultures at ALI showed similar replication kinetics in the bronchial cells.
(3) Clinical isolate-based HRSV-EMGFS5 replicates to higher viral titers than laboratory-adapted rHRSV in their of cotton rats and mice. Infection was predominantly present in diluted epithelial cells in cotton rats and in the olfactory mucosa of mice.

Results

(1) Epithelial cells from all anatomical locations were susceptible to HRSV infection, although subgroup A viruses disseminated and replicated faster than the subgroup B virus HRSV infected mostly ciliated epithelial cells in all cultures, leading to cilia degeneration.

(2) HRSV replication kinetics and cytopathic effect in 2D AO cultures at ALI were comparable with the commercially available bronchial cultures: both HRSV-A strains resulted in higher titers over time. Subgroup A viruses also had a replicative advantage in direct competition experiments.

(3) HRSV-EMGFS5 replicate to higher viral titers than rHRSV-EGFS5 in the upper respiratory tract of cotton rats and mice. Infection was mainly present in diluted epithelial cells in cotton rats and in the olfactory mucosa of mice.

Conclusion

(1) HRSV-A replicates faster than HRSV-B in cells from both the URT and LRT.
(2) HRSV infection in 2D AO cultures at ALI showed similar replication kinetics in the bronchial cells.
(3) Clinical isolate-based HRSV-EMGFS5 replicate to higher viral titers than laboratory-adapted rHRSV in their of cotton rats and mice. Infection was predominantly present in diluted epithelial cells in cotton rats and in the olfactory mucosa of mice.

In conclusion, using relevant in vitro and in vivo models we showed that HRSV replicates, already at early time points, in the entire respiratory tract. We also show that replication kinetics can vary significantly between strains and that HRSV tropism differs between mice and cotton rats.