Polarity of Influenza H5N1 Virus Infection in Respiratory Epithelial Cells and the Impact of Basolateral Release of Cytokines in Disease Pathogenesis

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Introduction

Highly pathogenic avian influenza virus (H5N1) is the first avian influenza virus that documented to cause respiratory disease and death in human. The biological basis for the severe human disease and high fatality rate remains unclear. We have previously demonstrated that when compared to human H1N1 and H3N2 influenza viruses, infection of influenza H5N1 virus led to the hyper-induction of pro-inflammatory cytokines in human primary macrophages and non-polarized respiratory epithelial cells in vitro. We also reported that patients with H5N1 disease have unusually high serum concentration of chemokines, e.g., IP-10. We therefore hypothesized that the differential hyper-induction of pro-inflammatory cytokines may contribute to the unusual severity of human H5N1 disease. In human, pathology of influenza A (H5N1) viruses are primarily respiratory pathogens targeting the respiratory epithelium, resulting in high level of cell death and tissue damage. As respiratory epithelial cells are primary target cells for replication of influenza viruses, a full understanding of how influenza virus interacts with respiratory epithelium is vital to advance our knowledge of its tropism and pathogenesis.

Methods

Primary human respiratory (bronchial and alveolar) epithelial cells were isolated and seeded onto collagen-coated transwell filters and cultured under air-liquid interface until cells were polarized and differentiated. The replication kinetic of influenza virus was assessed by virus yield titration ($TCID_{50}$) in MDCK cells. The cytokines expression profile induced by influenza H5N1 viruses (A/HK/483/97 and A/Vietnam/3046/04) was compared with human H1N1 virus by quantitative RT-PCR and ELISA.

Results

Polarized and differentiated bronchial and alveolar epithelial cells models are established for the study (Figure 1). We demonstrated that influenza A viruses (H5N1 and H1N1) can infect and productively replicate in the bronchial and alveolar epithelial cells leading to cytopathic effects. We found that the respiratory epithelial cells infected on the apical rather than the basolateral surface showed high levels of viral replication. Progeny influenza virus was released into the apical chamber at titers up to 3 logs higher than those recovered from the basolateral surface of polarized cell cultures (Figure 2). Influenza H5N1 viruses were more potent inducers of cytokines in human respiratory epithelial cells when compared to the H1N1 virus. We show that these cytokines are released preferentially at the basolateral aspect of the polarized epithelial cells (Figure 3).

Conclusion

Our data suggest that influenza H5N1 viruses enter and are released from the apical domain of respiratory epithelium, while the pro-inflammatory cytokines are released preferentially from the basolateral aspect of the respiratory epithelium. These finding may provide important insights into the mechanism of replication and pathogenesis of influenza H5N1 virus in humans.

Figure 1. Polarized and differentiated bronchial and alveolar epithelial cells cultured at air-liquid interface using transwell inserts.

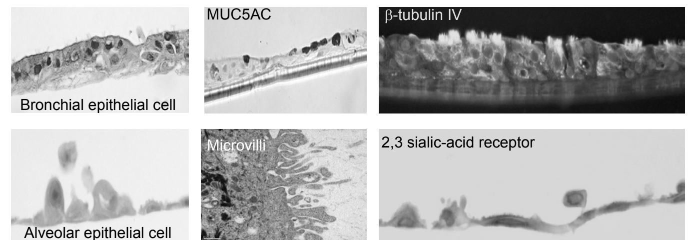

Figure 2. Apical entry and release of influenza H5N1 virus in human respiratory epithelium. The replication kinetic of influenza virus infected through the Apical (▪) and Basolateral (▫) surface of the respiratory epithelium was assessed by virus yield Titration ($TCID_{50}$/Ml) at 48 hours post infection.

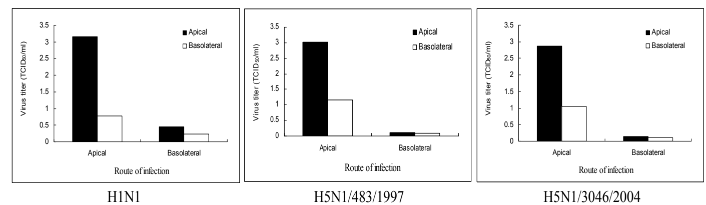

Figure 3. Basolateral release of proinflammatory cytokines induced by influenza (H5N1 and H1N1) viruses in human respiratory epithelium. IP-10 and RANTES were detected by ELISA from the cell supernatant collected from the Apical (A) and Basolateral (B) Surface of transwell inserts post infection.