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Angiopoietin-1 and keratinocyte growth factor restore the impaired alveolar fluid clearance induced by influenza H5N1 virus infection

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Background: Acute respiratory distress syndrome (ARDS) caused by high pathogenic avian influenza (HPAI) H5N1 virus infection has resulted in severe illness and high mortality rates among patients. Patients with ARDS are often characterized by impaired alveolar fluid clearance and alveolar edema. An understanding of the mechanism responsible for human alveolar edema will lead to the development of novel therapeutic treatments for ARDS patients. We hypothesized that the paracrine soluble factors angiopoietin-1 (Ang-1) and keratinocyte growth factor (KGF) can resolve alveolar fluid clearance by up-regulating the expression of major sodium and chloride transporters impaired by HPAI H5N1 virus infection. Materials and Methods: Human alveolar epithelial cells grown on transwell inserts were infected with HPAI H5N1 (A/HK/483/97) and low pathogenic avian influenza (LPAI) H1N1 (A/HK/54/98) viruses at MOI 0.1 or incubated with conditioned culture medium containing Ang-1 and/or KGF. At 24 and 48 h post-infection, the rate of alveolar fluid transport and protein permeability across the alveolar epithelium was measured. Protein expression of sodium and chloride transporters (Na-K-ATPase, CFTR, and epithelial sodium channel alpha subunit) was measured by qPCR, ELISA, and Western blot. Results: HPAI H5N1 (A/HK/483/97) virus infection significantly reduced net alveolar fluid transport and protein permeability when compared with H1N1 (A/HK/54/98) virus infection at 24 h post-infection and further reduced it at 48 h post-infection. This reduction in alveolar fluid clearance was associated with a substantial reduction in protein expression of Na-K-ATPase, CFTR, and epithelial sodium channel alpha subunit. The influenza virus–infected cells treated with Ang-1 and KGF restored the impaired alveolar edema fluid clearance and protein permeability after HPAI H5N1 virus infection. Furthermore, the paracrine soluble factors Ang-1 and KGF up-regulated the protein expression of the major sodium and chloride transporters resulting from the HPAI influenza virus infection. Conclusions: The paracrine soluble factors Ang-1 and KGF play an important role in maintaining human alveolar fluid clearance by up-regulating the sodium and chloride transporting systems in human alveolar epithelium. This study enriches the understanding of the development of ARDS in human H5N1 disease and may aid in the development of possible therapeutic applications.

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Immunomodulatory and anti-viral effects of statins in influenza H5N1 virus infection of human alveolar epithelial cells and peripheral blood–derived macrophages

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Background: Highly pathogenic avian influenza (HPAI) H5N1 virus panzootic in poultry continues to spread. It causes zoonotic human disease with a high (> 60%) fatality rate and continues to pose a pandemic threat. Based on clinical, animal, and in vitro cell studies, we and others have suggested that differences in viral replication competence, tissue tropism, and cytokine dysregulation between H5N1 and low pathogenic viruses may contribute to disease pathogenesis. Statins as HMG-CoA inhibitors act to reduce cholesterol and have been demonstrated to have anti-inflammatory and immune-modulatory activities. However, there is controversy about the benefits of statin use on influenza infection in mice and humans. In this study, we aimed to evaluate the effects of statin

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treatment in influenza infection using physiologically relevant in vitro models—human alveolar epithelial cells (AECs) and peripheral blood–derived macrophages (PBDMs). Materials and Methods: Primary human AECs and PBDMs were infected with HPAI H5N1 (A/HK/483/97) and seasonal H1N1 (A/HK/54/98) viruses in the presence or absence of statin (simvastatin and sevastatin) treatment. Virus replication was monitored by measuring infectious viral particles in cell culture supernatants using $TCID_{50}$. Immuno-modulatory effects of statins were examined by measuring the mRNA and protein expression of cytokines and chemokines using qPCR and ELISA. In order to understand the intervention of statins and influenza infection, the gene expression profile of selected members of the sterol-biosynthesis pathway in influenza virus–infected AECs and PBDMs were also monitored. The responses of a variety of cytokine treatments on the genes of the sterol-biosynthesis pathway were investigated in AECs. Furthermore, the intracellular free cholesterol level was also examined by enzymatic assay in AECs infected with influenza virus. Results: We demonstrated that both simvastatin and mevastatin exhibited a dose-dependent inhibition of influenza virus replication for both HPAI H5N1 and seasonal H1N1 viruses in human AECs and PBDMs. The observed inhibitory effect of simvastatin and mevastatin occurred below the non-specific toxic effects to cells, which were measured by MTT assay. Treatment of simvastatin and mevastatin significantly suppressed H5N1 virus–induced pro-inflammatory cytokines such as TNF-α in PBDMs and chemokines, including IP-10 and MCP-1 secretion in both AECs and PBDMs at 24 hours post-infection. We further showed that human AECs and PBDMs infected with both HPAI H5N1 and seasonal H1N1 viruses had significant down-regulation of sterol pathway gene expression at 24 hours post-infection. AECs and PBDMs treated with IFN-γ or IFN-β but not IL-1β, TNF, or IL-6, showed down-regulation of sterol pathway gene expression. In addition, we found that the free cholesterol level was significantly reduced at 24 and 48 h post-H5N1 virus infection in AECs and in IFN-β–treated AECs. These results further support a specific modulation of the sterol metabolic pathway upon influenza virus infection. Conclusions: Taken together, the controversy about the beneficial effects of statin use in influenza infection and our data suggest that statins possess both the antiviral and immune-regulatory effects in H5N1-infected in vitro cell models. We also demonstrated a highly specific response of AECs and PBDMs through a coordinated negative regulation of multiple sterol pathway members upon influenza virus infection or treatment of interferon. Identification of a reduction in sterol pathway gene expression and cholesterol levels with IFN treatment in human AECs offers new insights on the host-mediated antiviral responses through the sterol metabolism pathway and opens new therapeutic options for human influenza disease.

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Immune modulation by a novel narrow spectrum kinase inhibitor RV1088 as a therapeutic strategy for influenza virus infections

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Severe outcome following influenza infection has been linked to the over induction of the host innate immune response resulting a “cytokine storm.” In certain patient populations, a therapeutic approach to help control over induction of the innate immune response may be of benefit. However, concerns have been expressed that suppression of aspects of the host immune response might also lead to an increase in virus replication and directly enhance viral induced pathology. Using airway-liquid interface cultures of primary well-differentiated human airway epithelium (HAEs), we have demonstrated that a narrow spectrum kinase inhibitor, RV1088 (RespiVert Ltd.) can inhibit the induction of an array of human cytokines including IL6, IL8, IP10, and RANTES while at the same time not adversely increasing viral replication. In contrast, treatment of infected airway cells with fluticasone propionate, a steroid, did not inhibit any of the cytokine/chemokine responses measured. RV1088 also inhibited the viral induction of transcription from the interferon promoter acting at or below the level of MAVS. Used alone, RV1088 inhibited cytokine production by virus strains representative of all currently circulating subtypes and lineages of influenza A and B virus. Used in combination with Zanamivir, the virus titre released from HAE cells was suppressed even further than