Tropism of the novel human betacoronavirus lineage C virus in human ex vivo and in vitro cultures: potential transmissibility and pathogenesis in humans (abridged secondary publication)

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KEY MESSAGES

1. HCoV-EMC and SARS-CoV infected and productively replicated in human ex vivo lung and bronchus explants.
2. Non-ciliated bronchial epithelial cells, bronchiolar epithelial cells, and type I and type II pneumocytes are major targets for HCoV-EMC infection.
3. HCoV-EMC infection in bronchus and lung failed to induce interferon, with no inhibitory effect in suppressing HCoV-EMC infection by sialidase.

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Introduction

Coronavirus infections in humans are generally mild and self-limited. Until the outbreak of SARS in 2003, there was limited research on the tissue tropism and host response on human infection with coronaviruses. Compared with human MERS-CoV (HCoV-EMC), SARS-CoV was deficient at eliciting interferon (IFN)-β innate immune responses, because SARS-CoV encodes several antagonists of IFN sensing and signalling pathways. Tropism of SARS-CoV in the respiratory tract was primarily restricted to differentiated human airway epithelium and alveolar type II pneumocytes. In 2012, the novel HCoV-EMC was detected in two patients from Saudi Arabia and Qatar.[1] Thereafter, more cases were identified prospectively and retrospectively in Saudi Arabia, Qatar, and Jordan. Pneumonia leading to acute respiratory distress syndrome was the primary manifestation of the disease, but renal dysfunction was also observed in some cases. The World Health Organization provided a working case-definition of the disease.[2] The disease has an incubation period of up to 10 days and is not easily transmitted between humans. The Qatari patient had contact with camels and sheep.[3] Investigation (based on clinical disease and RT-PCR testing) of 64 contacts of the patient during his medical stay in the UK has not revealed secondary cases. The European Centre for Disease Prevention and Control reported an outbreak of severe respiratory illness in Jordan in April 2012, but no virological diagnosis was made. A number of these cases have been retrospectively studied, as has an outbreak of a respiratory illness in an intensive care unit in a hospital in Zarqa, Jordan. Seven nurses and one doctor were among the 11 affected, in which one of the nurses died who had underlying conditions. All cases had high fever and lower respiratory symptoms; only two of them were virologically confirmed to be caused by MERS-CoV.

We have previously used ex vivo cultures of human bronchus and lung explants tissues to investigate tropism of influenza viruses. Productive infection of the upper airways may correlate with the emergence of pandemic potential in swine influenza viruses. In the present study, we used ex vivo organ cultures of the human bronchus and lung to study the tissue tropism, virus replication kinetics, and innate immune response of the acute infection of HCoV-EMC, in comparison with SARS-CoV and (human coronavirus 229E) HCoV-229E. As some coronaviruses use 9O-Sia for virus entry,[4] HCoV-EMC may also use sialylated glycans as a receptor for binding and entry. We thus evaluated the effect of sialidase to examine if the removal of sialic acids can inhibit the infection with potential as a therapeutic option for patients with HCoV-EMC disease. Inhaled sialidase therapy options are currently in phase 2 clinical trials for influenza A virus.[5]

The aim of the study was to better understand the tissue tropism and pathogenesis of MERS-CoV in the human and to elucidate the possible therapeutic options for human infection of MERS-CoV.

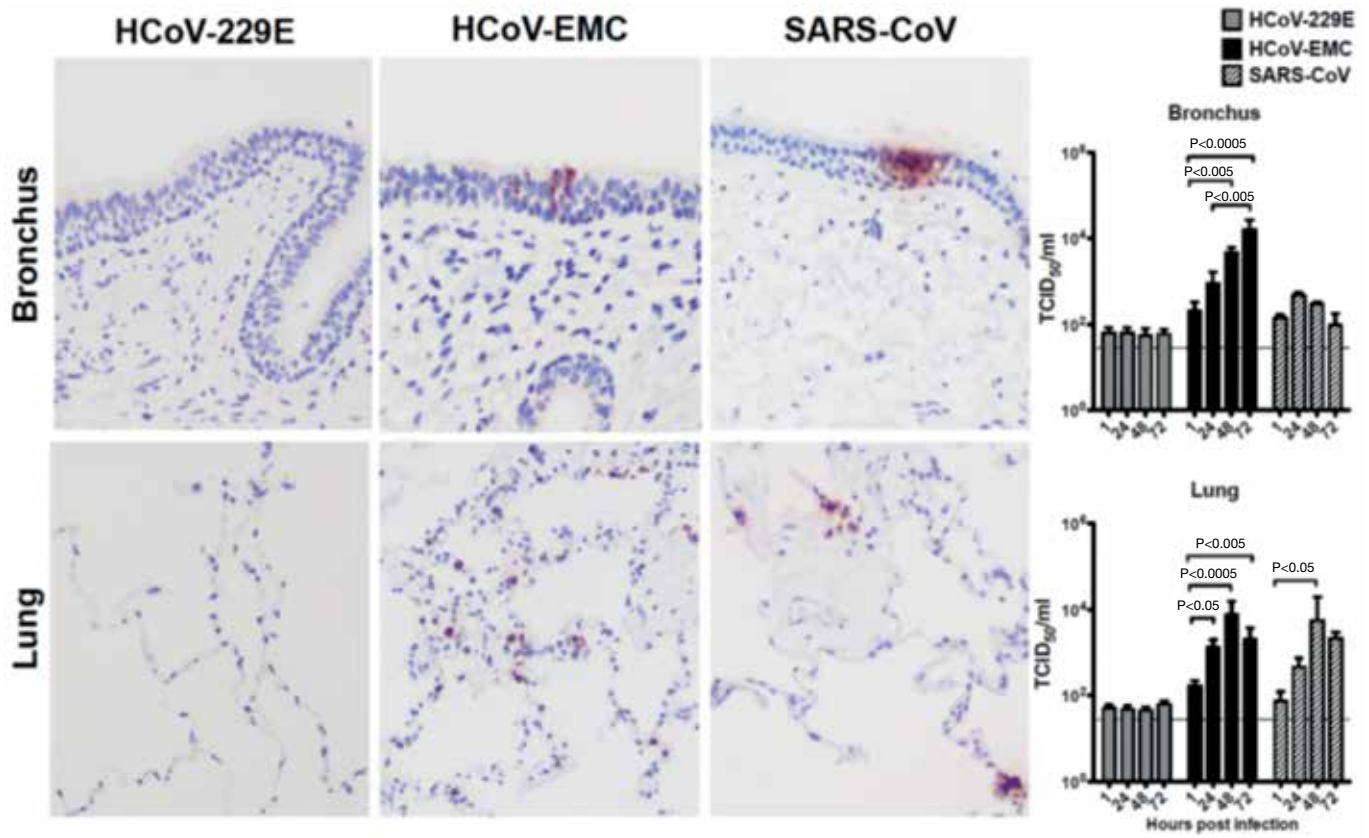

FIG 1. Tissue tropism of HCoV-229E, HCoV-EMC, and SARS-CoV in human bronchus and lung. At 24 hours post infection, tissue sections are stained using human coronaviruses N protein (reddish brown). Viral replication kinetics in ex vivo cultures of bronchi and lung biopsies infected with 10^6 $TCID_{50}$/mL of coronaviruses by virus titration at 37°C is shown.

Methods

We compared HCoV-EMC with SARS-CoV, HCoV-229E, and dromedary camel MERS-CoV in terms of tropism, replication competence, and host innate immune responses, using ex vivo explants cultures of human bronchus and lung, in vitro cell culture of Vero, and human respiratory epithelial cells. We also studied the effect of sialidase treatment of ex vivo human respiratory tissues and Vero cell on HCoV-EMC infection.

Results

HCoV-EMC infected and productively replicated in human ex vivo bronchus explants, whereas SARS-CoV infected bronchus explants and showed limited replication ability (Fig 1). HCoV-229E had no detectable infection or replication in bronchus explants. In ex vivo lung explants, viral antigen was not detected in HCoV-229E inoculated tissues, and no productive replication was observed. HCoV-EMC and SARS-CoV viruses extensively infected and replicated in lung explants.

In bronchus explants, we stained the ciliated cells and goblet cells using β-tubulin and MUC5AC, respectively (Fig 2). The infected cells (in red) were not co-localised with these two cell types and viral antigen was mainly found in non-ciliated bronchial epithelial cells. In lung, macrophages, epithelial cells, type I pneumocytes were stained with specific markers CD68, AE1/AE3, and podoplanin, respectively. HCoV-EMC did not co-stain with macrophages, but there was overlapping of staining with AE1/AE3 marker, which suggested that the target cells of HCoV-EMC infection were of epithelial origin and focal co-localisation was found in type I pneumocytes. Cellular morphology and immunohistochemistry for viral antigen (nucleoprotein) in the HCoV-EMC–infected lung tissues gave additional information on cell types targeted by HCoV-EMC at 48 hours post infection. Endothelial cells within the medium size interstitial vessels of the lung and bronchiolar epithelial cells were positive. Furthermore, we found extensive expression of cleaved caspase 3, an apoptosis marker, in ex vivo lung explants infected with HCoV-EMC and SARS-CoV but not in mock infected tissue. To investigate if the apoptosis was induced

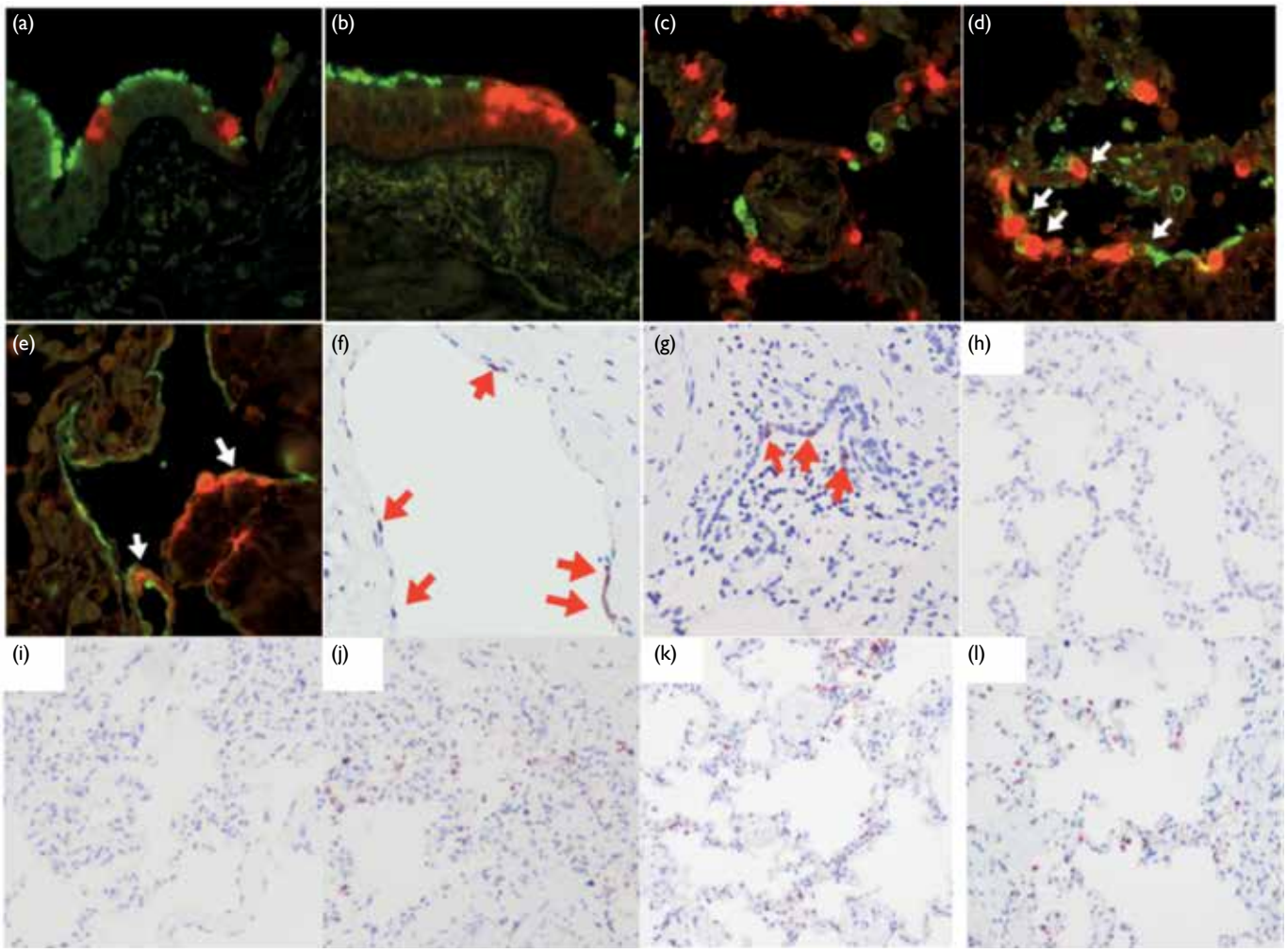

FIG 2. Cellular localisation of HCoV-EMC and induction of apoptosis in lung. HCoV-EMC stained in Vector Red (Red) in bronchus and lung tissue with cell marker conjugated with FITC (green). (a) B-tubulin (ciliated cell marker), (b) MUC5AC (goblet cell marker), (c) CD68 (macrophage marker), (d) AE1/3 (epithelial cell marker), (e) Podoplanin (type I pneumocyte marker) at 24 hours post infection. White arrows indicate cells with co-staining. (f) Cellular tropism of HCoV-EMC in lung, with human coronavirus N protein (stainedin reddish brown with red arrows) identified in endothelial cells at 24 hours post infection and in (g) bronchiolar epithelial cells at 48 hours post infection. Apoptotic cells are identified in the human lung ex vivo organ culture upon HCoV-EMC and SARS-CoV infection. Ex vivo culture of lung infected with (h) Mock, (i) HCoV-EMC, and (j) SARS-CoV at 48 hours post infection; reddish-brown stain identifies the presence of cleaved-caspase 3. Co-staining of (k) HCoV-EMC and (l) SARS-CoV antigen (pink stain) with cleaved-caspase 3 (reddish-brown stain).

directly by infection, we performed co-staining by immunohistochemistry of HCoV-EMC viral antigen (stained in pink) with cleaved caspase 3 (stained in reddish brown) using HCoV-EMC and SARS-CoV–infected human lung explants. Both HCoV-EMC and SARS-CoV–infected lung tissue revealed that the apoptotic cells were not the viral protein expressing cells suggesting that paracrine mechanisms may contribute to induction of apoptosis.

Ex vivo explants culture of bronchus and lung from three donors were infected with HCoV-EMC. Viral RNA was quantitated by RT-PCR. Host mRNA expression of type I (IFN-β) and type III (IL-29) interferons and cytokines and chemokines TNF-α and IP-10 was quantitated in HCoV-EMC or mock-infected bronchus and lung tissues. Viral gene expression increased by more than 2000-fold in bronchus and more than 180-fold in lung explants (Fig 3). However, compared to mock, HCoV-EMC infection in bronchus and lung failed to induce both IFN-β and TNF-α. There was marginal induction of IL-29 in virus-infected lung cultures at 48 hours post infection ($P<0.05$) and higher IP-10 mRNA expression at 24 hours post infection ($P<0.05$), compared with mock. IL-1β, MCP-1, MIP-1 alpha, MIP-1 beta, MIP-2 alpha, IL-6, IL-8, and RANTES mRNA were similarly quantified; no upregulation was detected in bronchus and lung explants.

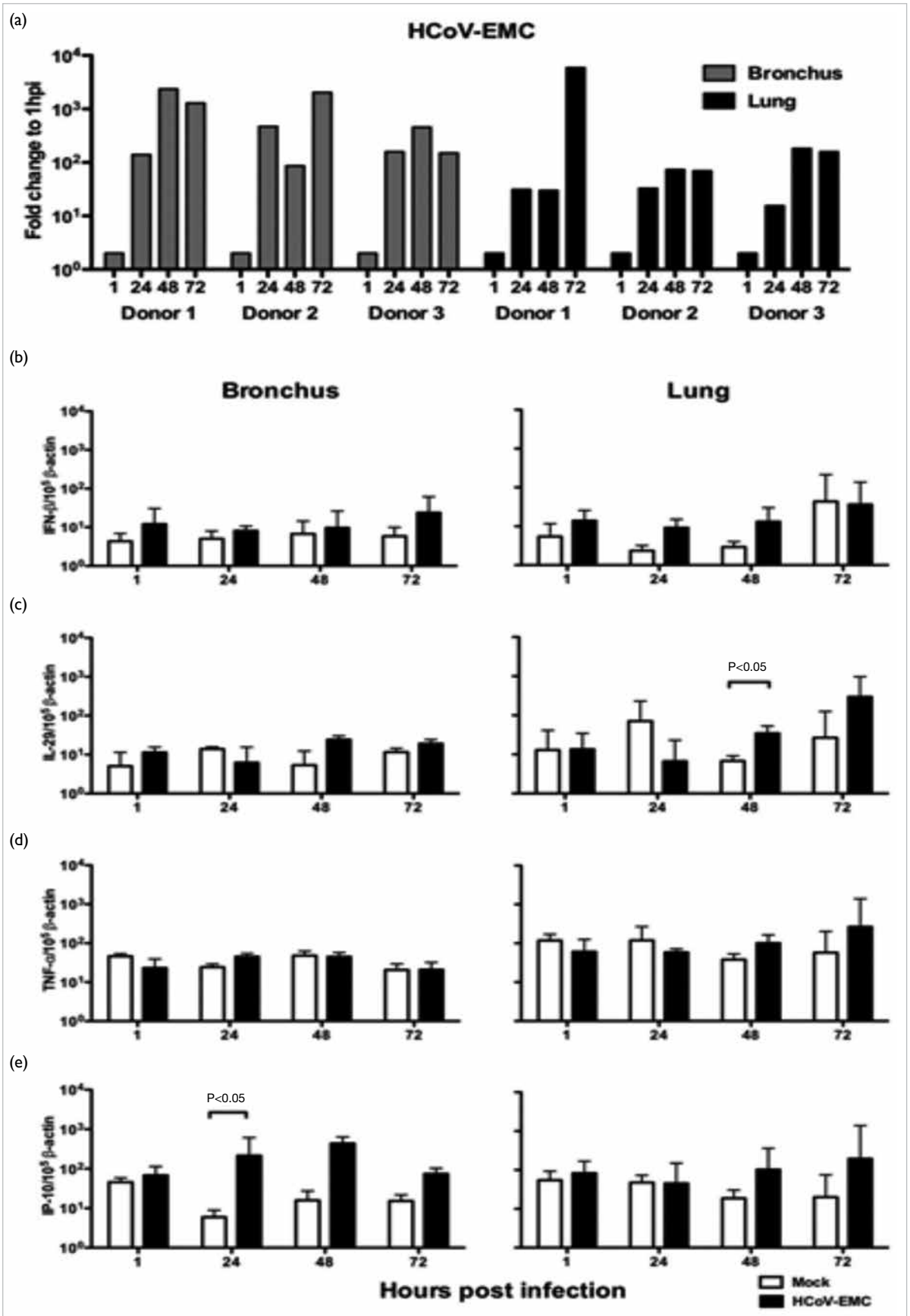

FIG 3. HCoV-EMC and cytokine and chemokine expression in ex vivo human bronchus and lung explants in response to infection. (a) Expression of HCoV-EMC genes at 1, 24, 48, and 72 hours post infection in ex vivo bronchial and lung tissues. (b to e) Expression (numbers of copies of mRNA per 10^5 β-actin copies) of IFN-β, IL-29, TNF-α, and IP-10 in mock-infected and infected ex vivo cultures at 1, 24, 48, and 72 hours post infection.

Discussion

Immunohistochemical analysis of virus-infected ex vivo lung cultures demonstrated that endothelial cells within interstitial blood vessels of the lung were targets for HCoV-EMC infection. This implies that HCoV-EMC may spread systemically to affect distant organs. Thus, renal dysfunction in some patients with HCoV-EMC may be due to virus dissemination to the kidney. Further clinical studies are needed to address whether viral dissemination occurs and whether the renal dysfunction is due to viral invasion of the kidneys.

Immunofluorescence study and transmission electron microscopy of infected ex vivo cultures of lung and bronchus enable further define the cell types targeted by HCoV-EMC. Non-ciliated bronchial epithelial cells, bronchiolar epithelial cells, and type I and type II pneumocytes appear to be the major target for HCoV-EMC infection. We did not observe virus-infected alveolar macrophages in ex vivo lung cultures. Preliminary study on peripheral blood monocyte-derived macrophages did not support the replication of the HCoV-EMC. Human and dromedary MERS-CoV have comparable tropism and replication competence in human respiratory ex vivo cultures. This is the first report of comparative data on living human respiratory tissue in situ. Therefore, our study provides novel and crucial information to the understanding of a disease with global public health significance.

Some betacoronaviruses in lineage A (eg, HCoV-OC43) possess an esterase and use 9 O-acetylated Sia as virus entry receptors.[4] Inhalational therapy with a sialidase may provide some clinical benefit. We had previously shown an antiviral effect of the sialidase DAS181 in ex vivo lung cultures infected with influenza viruses. However, no antiviral effect was observed in suppressing HCoV-EMC infection in lung and bronchus explants and Vero cells. This suggests that HCoV-EMC is not dependent on a sialylated receptor for entry into cells and virus replication. This is in agreement with the genomic information of HCoV-EMC suggesting that the virus does not contain esterase, which is present in HCoV-OC43 and is important in cleaving sialic acids and promoting its progeny virions to spread.

Conclusion

This study illustrates the clinical utility of ex vivo cultures of the human respiratory system to investigate newly emerging respiratory viruses. There have been no autopsy reports describing the virus-induced pathology in the lung to confirm our findings. Nonetheless, autopsy data often reflect the late-stage disease in patients who may have been on mechanical ventilation for long time. Thus, studies of ex vivo experimental infection of the human respiratory tract are invaluable to understand virus tropism and pathogenesis as well as to provide evaluation of potential therapeutic options.

As current antiviral therapy is not effective and specific against coronaviruses, the potential therapeutic benefit of sialidase (DAS181) treatment, which is in phase II clinical trial, is an important research.

Funding

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Disclosure

The results of this research have been previously published in:

1. Chan RW, Chan MC, Agnihothram S, et al. Tropism of and innate immune responses to the novel human betacoronavirus lineage C virus in human ex vivo respiratory organ cultures. J Virol 2013;87:6604-14.

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