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Pathogenesis of SARS coronavirus infection using human lung epithelial cells: an in vitro model

Introduction

Severe acute respiratory syndrome (SARS) affected more than 8000 patients in over 32 countries. One third of the patients spontaneously recovered early in the course of disease; others progressed and about 30% needed intensive care. The aetiological agent was the SARS-coronavirus (SARS-CoV).1,2 Experimental infection of cynomologous macaques with SARS-CoV reproduced a disease with diffuse alveolar damage and multi-nucleate syncytia reminiscent of human SARS.3 The SARS-CoV has been demonstrated by reverse transcriptase polymerase chain reaction (RT-PCR) and virus isolation in respiratory secretions, faeces, urine, and lung biopsy, indicating that SARS-CoV is a disseminated infection rather than being restricted to the respiratory tract.

The virus antigen can be demonstrated in pneumocytes of SARS patients dying within the first 10 days of illness.4 The predominant cells infiltrating the alveoli are CD68-positive cells. This suggests that alveolar pneumocytes may be the target cells of SARS-CoV and play a role in the pathogenesis of the disease. The SARS-CoV cannot replicate in differentiated respiratory epithelial cells or in primary non-differentiated bronchial and alveolar epithelial cells, even when its functional receptor—ACE2—is present (unpublished data).5 It is therefore important to study virus-cell interactions in primary lung alveolar epithelial cells.

We have established differentiated models of human bronchial epithelial cells and have isolated the differentiated status of human types I and II pneumocytes. An ex vivo model for lung culture was also established to address the tropism and pathogenesis of SARS-CoV in human respiratory epithelial cells.

We aimed to establish (1) an in vitro model for primary human alveolar types I and II epithelial cells (pneumocytes), (2) an in vitro model for well-differentiated bronchial epithelial cells, and (3) an ex vivo model for SARS-CoV-infected human lung tissue culture. In addition, we aimed to define the gene expression profile (cytokine, chemokine) of primary human respiratory epithelial cells infected with SARS-CoV and compare it with human coronavirus 229E and influenza A (H1N1) virus.

Methods

This study was conducted from January 2006 to December 2007. Primary human respiratory epithelial cells were infected with SARS-CoV in vitro. Virus replication was monitored by measuring the levels of the SARS nucleocapsid genes, by immunofluorescence detection of the SARS-CoV nucleoprotein, and by titration of the infectious virus. The gene and protein expression profiles (cytokine and chemokine) of respiratory epithelial cells infected with SARS-CoV, human coronavirus 229E, and influenza A (H1N1) virus were compared using real-time quantitative RT-PCR.

Results

To evaluate SARS-CoV infection in human respiratory tract, ex vivo models for human lung culture and in vitro models for well-differentiated and polarised...
human respiratory epithelial cells representing the bronchial and alveolar epithelial cells were developed (Fig 1). The tropism of some emerging infectious respiratory viruses (e.g., SARS-CoV, avian and human influenza viruses) was investigated using these cultures. The SARS-CoV initiated viral gene transcription and protein synthesis in well-differentiated bronchial epithelial cells only (Fig 2) and showed a productive replication in vitro. In well-differentiated bronchial epithelial cells, type I interferons (interferon-beta) and cytokine and chemokines (e.g., RANTES, IP-10, IL-6, IL-8, MCP-1, MIP-1α, MIP-2α) were markedly induced after infection with SARS-CoV, compared to human coronavirus HCoV229E and influenza A (H1N1) virus (Fig 3). However, SARS-CoV was unable to infect the alveolar type I-like pneumocytes, but there was a very limited infection and abortive productivity replication of this virus in type II pneumocytes (Fig 1). In an ex vivo experiment for lung culture infection, only the alveolar macrophages were infected with SARS-CoV, whereas alveolar epithelial cells were uninfected.

![Image](image-url)

**Fig 1.** Alveolar (a) type I-like and (b) type II pneumocytes infected with SARS-CoV in vitro by H&E staining. (c) ACE2 mRNA expression of the alveolar type I-like and type II pneumocytes in vitro from day 0 to 21 in culture. (d) Immunofluorescence staining of type II pneumocytes 24 and 48 hours post infection with SARS-CoV and mock infection. The SARS-CoV nucleoprotein was stained green with FITC-conjugated mouse anti-NP protein antibody. (e) The virus titre of the supernatant collected from SARS-CoV–infected type II pneumocytes from 1 to 144 hours post infection
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Fig 2. Well-differentiated normal human bronchial epithelial (NHBE) cells in vitro cultured in ALI for 28 days
(a) ACE2 mRNA expression of the well-differentiated NHBE cells from day 0 to 28 in air-liquid interface culture. (b) Immunofluorescence
staining of well-differentiated NHBE cells 48 and 120 hours post infection with SARS-CoV and mock infection. The SARS-CoV
nucleoprotein was stained green with FITC-conjugated mouse anti-NP protein antibody. (c) The virus titre of the supernatant collected
and the mRNA expression of SARS-CoV nucleocapsid gene from SARS-CoV–infected well-differentiated NHBE cells from 1 to 168 hours
post infection

Conclusions

Type II pneumocytes and well-differentiated bronchial epithelial cells can be infected with SARS-CoV but in vitro productive infectivity was not shown with alveolar type I-like pneumocytes and non-differentiated bronchial epithelial cells. The in vitro model enables a better understanding of how human respiratory epithelium responds to SARS-CoV and other respiratory viruses in order to better deal with other emerging infections such as avian influenza H5N1 virus. It also helps elucidate the underlying mechanism of inter-species transmission of animal pathogens to humans. Besides, SARS-CoV showed limited tissue tropism for human respiratory tract in vitro (infection and replication demonstrated only in well-differentiated bronchial epithelial cells). Therefore, it is important to use the appropriate in vitro human respiratory model to study the pathogenesis of SARS-CoV.

The host innate immune response induced by the SARS-CoV infection can elicit strong induction of an IFN response in human well-differentiated bronchial epithelial cells. In contrast to the low pathogenicity of HCoV 229E and influenza H1N1 viruses, SARS-CoV can trigger the release of certain cytokines and chemokines in respiratory epithelium. The differential hyper-induction of cytokines plays an important role in human infection with SARS-CoV. Comparison of the gene expression profile of SARS-CoV with the profiles of other highly pathogenic viruses (such as H5N1) may provide further insight into host molecular mechanisms involving viral pathogenicity.

Implications

In vitro and ex vivo studies of the tissue tropism of SARS-CoV can be extended to other emerging respiratory viruses that target human respiratory epithelial cells, especially H5N1 and/or H1N1 viruses. Such studies can be used to characterise and isolate emerging respiratory viruses that are difficult to culture in the laboratory (eg coronavirus HKU1) using transformed cell lines.
Fig 3. The cytokine and chemokine mRNA expression profile, IFN-β, IP-10, RANTES, MCP-1, MIP-1α, MIP-2α, IL-6, and IL-8 gene expression of the well-differentiated normal human bronchial epithelial cells 0, 24, 48, and 72 hours post infection of SARS-CoV, HCoV 229E, and influenza H1N1 virus. The means and standard errors of three representative experiments are shown.
The host innate immune response–related gene expression profiling of respiratory epithelial cells infected with SARS-CoV has provided information about a group of cytokine and chemokine genes that may be involved in its pathogenicity. Our in vitro and ex vivo models enable studying the tropism and pathogenesis of SARS-CoV, especially when suitable small animal models adhering to the 3R principles on the use of animals in research are lacking.

Our study focused on one key component of the innate immune response—the respiratory epithelium. Further investigations of this hypothesis with relevant animal models of SARS could also be considered. In vitro, the SARS-CoV is highly susceptible to the antiviral effects of IFN-ß. In studies on primates, IFN therapy (in combination with steroids) is beneficial for both prophylaxis and early therapy. Our results may provide a biological basis for the observed therapeutic benefit with IFNs in SARS patients when administered early.

Acknowledgement

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References