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Halting SARS-CoV-2: lung organoids step up to the plate

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Defining the pulmonary cell types infected by SARS-CoV-2 and finding ways to prevent subsequent tissue damage are key goals for controlling COVID-19. Recent work establishing a human lung organoid-derived air–liquid interface model permissive to SARS-CoV-2 infection identifies alveolar type II cells as the primary cell type infected, reports an infection-induced interferon response and demonstrates the effectiveness of interferon lambda 1 treatment in dampening lung infection.

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See also: **MM Lamers *et al*** (March 2021)

Coronavirus disease-2019 (COVID-19) has triggered a global pandemic and is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This novel coronavirus causes a spectrum of respiratory illnesses ranging from mild upper airway disease to life-threatening acute respiratory distress syndrome (ARDS), placing the lung as a primary organ to study in the worldwide effort to control this deadly respiratory virus. Within the lung, epithelial cells are the primary cell types that express ACE2 and TMPRSS2, a receptor and a protease essential for viral entry, respectively (Wang *et al*, 2020). However, how much of their expression level dictates cellular susceptibility to infection remains unclear. At the molecular level, it has been shown that SARS-CoV-2 infection induces interferon stimulated gene (ISG) expression. Addressing whether this upregulation occurs in infected cells or bystander cells and defining the cascade of molecular changes that follow is critical for devising prophylactic strategies to prevent severe infection.

Animal models such as transgenic mice expressing human *ACE2* and golden hamsters have been used to study COVID-19, but none was able to recapitulate severe lung infection. This elevated the need to directly use human lung cells, especially non-immortalized cell lines, to simulate *in vivo* infection. Several human lung cell culture platforms have been developed. A workhorse system is air–liquid interface (ALI) culture where airway progenitors such as basal cells efficiently differentiate into club, goblet and ciliated cells, recapitulating an *in vivo* airway organization (Karp *et al*, 2002). Primary epithelial cells can also be cultured as three-dimensional (3D) organoids, forming bronchospheres or alveolospheres, depending on the source of the epithelial cells (Barkauskas *et al*, 2017). Co-culture with mesenchymal cells further improves the authenticity of the microenvironment and differentiation efficiency. The drawbacks of these primary cell-based systems are the challenge to obtain human lung tissue and wide donor-to-donor variation, in some cases due to underlying disease state. Although 3D organoids more closely recapitulate *in vivo* signalling, the apical side is oriented towards the centre, thus making it difficult to be used for infection studies. Lung cells derived from pluripotent stem cells (PSCs) including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have allowed investigators to mass produce, store and genetically manipulate human lung cells (Leibel *et al*, 2020). The primary challenge remains the immaturity of the lung cells generated.

In a recent study, Lamers *et al* (2021) adapted a 3D lung organoid culture system based on multi-potent human distal tip cells from fetal lungs (Nikolić *et al*, 2017). These cells were expanded as submerged organoids and then changed to air–liquid interface to differentiate, further stimulated by niche signals from mesenchymal cells in the bottom compartment. Markers for alveolar type 1 and type 2 cells as well as for airway cells increased, demonstrating formation of mixed bronchioalveolar cell types. The differentiated cells also expressed ACE2 and TMPRSS2, setting the stage for infection (Fig 1).

Infection by SARS-CoV-2 was achieved at a low multiplicity of infection (MOI) of 0.01 and 0.1, suggesting that these cells are readily infected. Most of the infected cells were alveolar type 2 cells, while airway epithelial cells such as pulmonary neuroendocrine cells or lung mesenchymal cells were not infected. The demonstrated susceptibility of alveolar cells to SARS-CoV-2 infection is notable, as COVID-19-associated ARDS is a pathological condition of the alveolar region, where vital gas exchange occurs.

Bulk RNA-seq showed an increase of ISG signature following infection. Interestingly, staining for phosphorylated STAT1, a downstream readout of interferon activation, showed an increase of signal in the bystander cells, but not in SARS-CoV-2 infected cells. This result suggests that attention should be paid to bystander cells to address downstream interferon response.

To test whether the bronchioalveolar system can be used for SARS-CoV-2 drug screens, Lamers *et al* (2021) investigated the effect of Interferon lambda 1 (IFN-λ1) in

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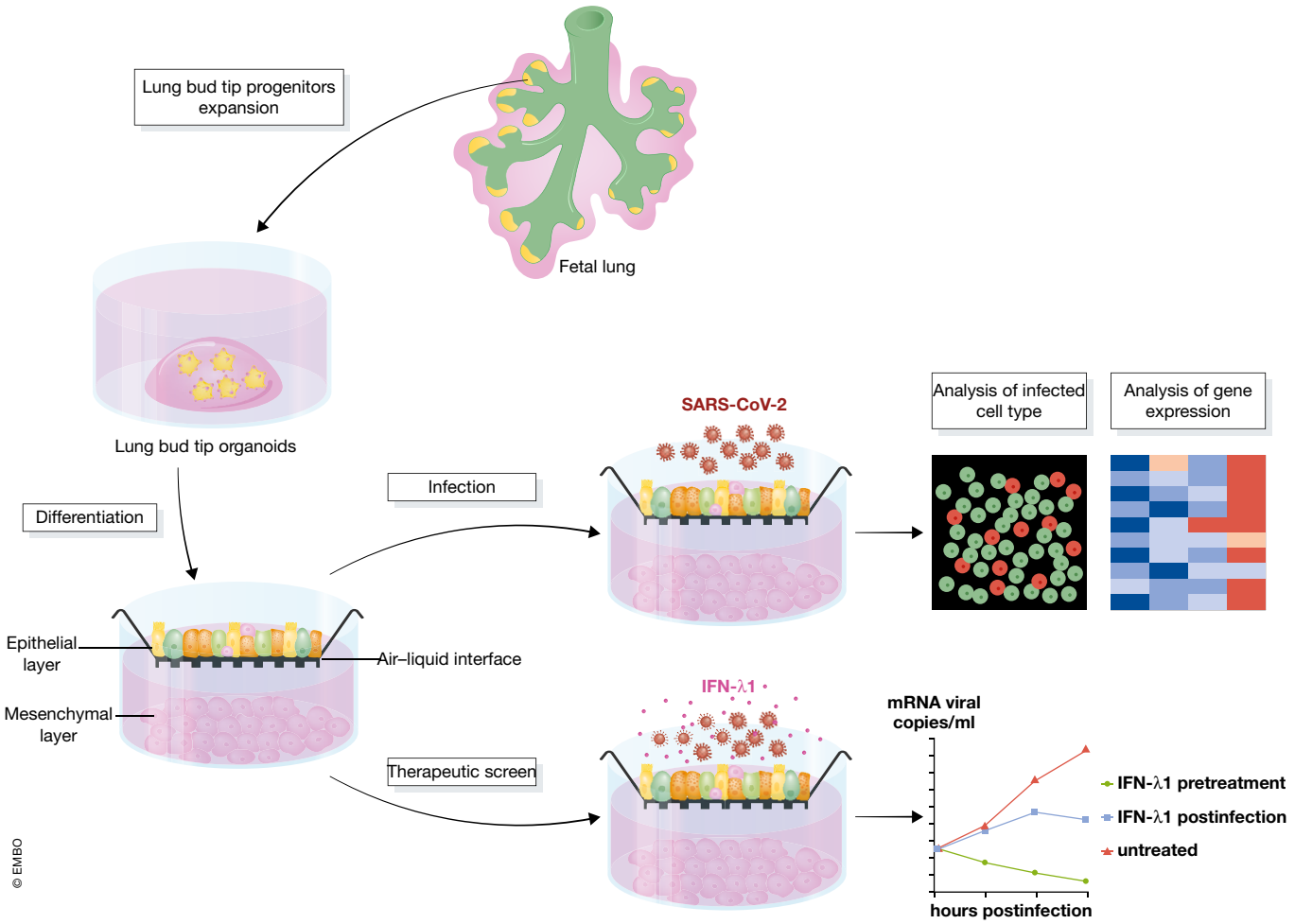

Figure 1. A bronchioalveolar lung culture platform for the study of SARS-CoV-2 lung infection.

This diagram illustrates the bronchioalveolar culture system established in Lamers *et al* (2021). Cells in this culture, in particular alveolar type cells, can be efficiently infected by SARS-CoV-2. Infection led to an increase in ISG expression. Treatment with IFN-λ1 either before or after infection abrogated or reduced infection rate, respectively.

modulating infectability. Treatment of cells 2 h prior to infection completely aborted viral replication. Treatment of cells 24 h after infection reduced infectious virus by ~ 5 logs and virus RNA copies by ~ 3 logs, demonstrating the antiviral activity of IFN-λ1 and the value of the bronchioalveolar system in drug screens.

Other human lung cell culture platforms have recently been developed to study SARS-CoV-2 infection. For example, primary airway cells in air–liquid interface culture were infected with a high viral load and confirmed infection of ciliated and goblet cells (Hao *et al*, 2020). Cigarette smoke exposure led to goblet cell metaplasia and a higher infection rate (Purkayastha *et al*, 2020). Primary lung-derived alveolospheres were infected and mirrored features of COVID-19 lungs including increased interferon, pro-inflammatory, and apoptotic pathway genes and decreased surfactants (Katsura *et al*, 2020; Youk *et al*, 2020). iPSC-derived alveolar type 2 cells adapted to air–liquid interface culture were infected and showed an inflammatory phenotype with upregulation of NFkB signalling and loss of mature alveolar program (Huang *et al*, 2020). The iPSC-derived alveolar type 2-like cell cultures also offered an ideal platform for high-throughput chemical screening (Han *et al*, 2020).

The human lung cell platforms, including the one established here, open opportunities for in-depth studies of SARS-CoV-2 infection of the lung. For example, having airway luminal cells such as club, ciliated and goblet cells in the same mixed culture with alveolar type 1 and type 2 cells will enable testing of relative infectability of lung epithelial cells, thus allowing assessment of the kinetics of SARS-CoV-2 lung infection once the virus is inhaled through the airway. Analysis of bulk RNA-seq data across multiple time points will chart the time course of molecular impact. Application of single-cell RNA-seq to the system will tease out the specific contribution of infected versus bystander cells to inflammatory and antiviral signals. Building on cultures such as the bronchioalveolar system used here, addition of immune cells and endothelial cells will allow testing of how the 3D alveolar and airway units respond to SARS-CoV-2 infection. We are hopeful that with vaccinations, COVID-19 will be better controlled. Once this is achieved, screens for therapeutics will be re-directed to preventing

and reversing lung damage caused by infection. Towards this goal, lung progenitor cultures such as the one established in this study (Lamers *et al*, 2021) will remain a pivotal tool in the search to repair chronic respiratory dysfunction following infection by not just SARS-CoV-2, but also other respiratory pathogens.

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