INTRODUCTION

There has been a recent shift away from both in vivo and transformed cell line models and towards advanced primary-derived human in vitro culture systems. Advanced cell models, such as airway organoids and epithelial immune co-cultures, could serve as powerful platforms to study viral infection and pathogenesis. Despite the advancements in human primary-derived in vitro models, limitations still remain. Among the challenges of accessing the apical surface of epithelia in traditional organoids with basal side-out polarity, as well as the lack of heterogeneity and paracrine signaling comes to prominence. To overcome these hurdles, we developed the two different human lung culture systems and their use in host-pathogen interaction studies and in drug screening applications (Figure 1).

1. Co-culture model with macrophages

We developed a workflow to generate complex co-cultures of human bronchial epithelial cells (HBECs) and blood-derived macrophages. HBECs may be seeded into a semi-permeable cell culture insert and placed at the air-liquid interface. Exposure to air facilitates recapitulate aspects of airway physiology, and these cultures are amenable to viral infection. Since the system has the potential to more accurately recapitulate the in vivo microenvironment, we assessed the effect of M0-like, M1-like, and M2-like macrophages following respiratory syncytial virus infection.

2. Aplical-out airway organoids for drug screening

The establishment of an air-liquid interface culture relies on the use of permeable inserts which has limited scalability. In traditional extracellular matrix (ECM)-embedded airway organoids, the apical side facing inward. To address these limitations, we developed a novel ECM-free, apical-out airway organoid (AOAO) model that enables easy access to the apical surface. This model organoids are amenable to viral infection and were used in an antiviral drug assay.

METHODS: Co-culture and infection

HBECs and Human blood-derived monocytes were isolated using EasySep™ Monocyte Isolation Kit and cultured in ImmunoCult™-SF Medium or after co-culture. Macrophage phenotype was confirmed by expressing M1-like marker CD80, and that macrophages. TER measurements for HBECs were not significantly affected by observed after co-culture. Data represent mean ± SEM (n = 4 - 5 donors). Scale bar = 1 mm.

RESULTS: Co-culture and infection

The two different human lung culture systems used to study viral infection are different cell culture models. RSV infection was potentiated in M0 and M2 co-cultures maintained in ALI medium compared to M0 or M2 media. Infection was observed in co-cultures of M0 in IMM and co-cultures of M2 in M2 medium for 72 hours. Infection was observed in co-cultures of M2 in IMM and co-cultures of M2 in M2 medium for 72 hours. No significant changes were observed after co-culture. Data represent mean ± SEM (n = 4 - 5 donors).

METHODS: AAOA Culture and infection

Generation of AAOAOs: HBECs were cultured in PneumaCult™-Ex Plus Medium in 2D, dissociated into single cells, resuspended in PneumaCult™-ALI Medium and seeded on 100 ECM-free semi-permeable inserts in 24-well plates. HBECs were cultured in ALI Medium for 4 weeks. After 4 weeks of culture, the inserts were assembled to form an air-liquid interface. HBECs in Transwell® inserts were placed on top of differentiated macrophages at the bottom of the lower compartment in either expanded in PneumaCult™-Ex Plus and subsequently differentiated in PneumaCult™-ALI (ALI) medium. After 4 weeks of differentiation at the air-liquid interface, HBECs in Transwell® inserts were either exposed to naïve HBECs or M1-like macrophages. Infection was potentiated in M0 and M2 co-cultures maintained in ALI Medium compared to M0 or M2 media, indicating that the choice of ECM-free, defined, and scalable workflow.

RESULTS: AAOA Culture and infection

Aplical-out airway organoids have Outward Facing Actively Beating Cilia and Express Expected Airway Ciliated Cells Similar to Air-Liquid Interface Cultures.

To determine the effect of antiviral compounds on the viral load of enterovirus D68-infected AAOAOs, 2.5 μM rupintrivir or 5 μM itraconazole was added to the cultures at 0 hours post-infection. 100 μL of medium was collected to detect viral RNA load at 24, 48, and 72 hours post-infection.

METHODS: AAOAO Culture and Infection

(A) Successful viral infection occurred in AAOAOs using RSV and in other differentiation and infection systems used to study viral infection and pathogenesis. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection. Successfully modeled the antiviral effect of rupintrivir and itraconazole in viral RNA load and CPE following enterovirus D68 infection.