EDITORIALS

Human Lung Slices: New Uses for an Old Model

Even before the coronavirus disease (COVID-19) pandemic, respiratory infections were the third leading cause of death worldwide (1). Their presence exacerbates other respiratory diseases, including asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis (2, 3), whereas lung diseases like COPD or asthma facilitate pulmonary infection because of a partially disrupted airway epithelium (3).

The target cell of most respiratory viruses is the epithelial cell, which is the first and essential barrier against viruses in the respiratory tract. The infection causes an acute immune response, cell differentiation, regeneration of the epithelium, and later resolution. On the one hand, infection experiments have been predominantly performed *in vivo* on laboratory animals—mostly mice and rats—displaying the most complex systems. The obtained results still need to be translated to the human organism. On the other hand, the infection of airway epithelial cells *in vitro* provides a simple and robust model, mainly using primary human cells or cell lines. Primary epithelial cells respond to different surface conditions, also depending on their origin, by rapid differentiation and variation of their phenotype (4). As there are no other cell types present, part of the classical immunological response is missing. The growing evidence underscoring the importance of intra- and intercellular communication, as well as cell–matrix interactions (4, 5), has led to the development of more complex *in vitro* (ex vivo) models over the last few years. Their complexity bridges the gap between *in vivo* and single cell cultures, as they are of lower complexity than *in vivo* and yet more complex compared with single cell cultures. Furthermore, the different complexities also affect the role and interaction of the viruses with the respiratory tract. Models incorporating increased complexity include classical coculture (two-dimensional), organoid culture, differentiated multilayer epithelial cells grown at an air–liquid interface, lung on a chip, and precision-cut lung slices (PCLS) or lung explants. PCLS include all resident cell types, including T cells and macrophages, and also permit assessments of functional properties (6). Lung slices are used in a broad spectrum of research, such as toxicology, immunology, and pharmacology, as well as bacterial and viral infection models (7–9). Also, human lung slices are of particular value for translational research despite their variability of responses.

In this issue of the *Journal*, Wronski and colleagues (pp. 544–554) describe a study in which they induced stable infection with rhinovirus (RV) in PCLS, validated the replication of the virus, and determined differences in genomic and release of some mediators (10). Even though an incubation time of three days only captures the early stage of infection, Wronski and colleagues clearly demonstrate a typical type I IFN-driven viral response (11). They were able to discriminate changes in gene expression of RV-infected PCLS, which they clustered depending on cell types or signaling pathways. Replication-independent changes induced by inactivated viruses showed different patterns of gene expression compared with active viruses. Most importantly, they found an overlap of strongly induced or suppressed genes of the RV-infected PCLS compared with genomics of brushed primary epithelial cells from patients with asthma and COPD available in a public database (12, 13). Interestingly, these RV-induced gene expressions for asthma and COPD differed substantially, with only a small overlap between the two groups. Finally, the antiviral treatment of human PCLS by Rupintrivir affected solely the gene regulation of inflammation and not the integrity and healing of the epithelium. This allows for the discrimination of drug action at different stages of the infection process and correlates it to distinct gene regulation patterns. Thus, it permits better insight into mechanisms of drug actions and their consequences.

There are some limitations and possible improvements for further research to be discussed. First, the number of patients used for the genomic analysis is relatively small (3–4 patients). Wronski and colleagues referred to the small interpatient differences, but an additional direct comparison of each condition per patient would be helpful in statistical analysis. More details on medical history, such as diseases and premedication, would further strengthen the conclusions. These factors should have an impact on the results of genomic analysis. The use of human PCLS obtained from patients with COPD or asthma followed by a viral infection could elucidate factors and pathways of exacerbation. The production of human lung slices can lead to the availability of a large number of PCLS as well as functional cryopreserved PCLS (2). Even though the number of viable cells is slightly reduced, thawed PCLS may still be useful for infection experiments.

Second, during the slicing process of PCLS, growth factors responsible for wound healing in the tissue are released and induced. In order to distinguish gene inductions caused by the slicing process from the former, the genetic data should be compared to native human lung tissue, which is also available in public databases.

Third, human PCLS with small airways, as used by Wronski and colleagues, show changes in gene expression and mediator release. This should be compared to possible physiological changes in the same tissue, predominantly airway contraction (14), ciliary beating, and mucus release. This combination would give deeper insight and possibly yield molecular, immunological, and functional findings.

To ameliorate the immunological response in PCLS, methodological improvements would be necessary. The infection process involves local and systemic immune responses, which implies chemotaxis of immune cells. PCLS allow the study mainly of local immune responses, which lack interaction with the circulating immune cells. Cultivation of PCLS in an air–liquid interface and addition of distinct immune cells could yield crucial insight into chemotaxis.

Infections by viruses, such as respiratory syncytial virus, enterovirus, influenza virus, adenovirus, and coronaviruses (15) are

responsible for triggering exacerbations of asthma and COPD (2, 3). Even though RVs are the most prominent, this model of human PCLS should be extended to other viruses. Most importantly, viruses for which infection in other mammals is difficult (like severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] in mice [16]) should be considered. However, infection with parainfluenza virus in PCLS infects cells beneath the epithelium, which can be accessed by the virus after the slicing process. This is clearly a methodological artifact in PCLS and shows a limitation of the method (15).

In addition, multiple infections often occur simultaneously in patients, and different viruses affect different epithelial cell types. Therefore, the combination of viruses and/or bacteria would be important.

In conclusion, the manuscript's in-depth analysis of viral responses is of great methodological importance and a basis for future research. This is crucial for the study of infectious diseases and the treatment of lung diseases. ■

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References

1. Shah T, Press VG, Huisingh-Scheetz M, White SR. COPD readmissions: addressing COPD in the era of value-based health care. *Chest* 2016; 150:916–926.
2. Ortega H, Nickle D, Carter L. Rhinovirus and asthma: challenges and opportunities. *Rev Med Virol* 2021;31:e2193.
3. Linden D, Guo-Parke H, Coyle PV, Fairley D, McAuley DF, Taggart CC, *et al.* Respiratory viral infection: a potential "missing link" in the pathogenesis of COPD. *Eur Respir Rev* 2019;28:180063.
4. Whitsett JA, Kalin TV, Xu Y, Kalinichenko VV. Building and regenerating the lung cell by cell. *Physiol Rev* 2019;99:513–554.
5. Schiller HB, Montoro DT, Simon LM, Rawlins EL, Meyer KB, Strunz M, *et al.* The human lung cell atlas: a high-resolution reference map of the human lung in health and disease. *Am J Respir Cell Mol Biol* 2019;61: 31–41.
6. Liu G, Betts C, Cunoosamy DM, Åberg PM, Hornberg JJ, Sivars KB, *et al.* Use of precision cut lung slices as a translational model for the study of lung biology. *Respir Res* 2019;20:162.
7. Wohlsen A, Martin C, Vollmer E, Branscheid D, Magnussen H, Becker WM, *et al.* The early allergic response in small airways of human precision-cut lung slices. *Eur Respir J* 2003;21:1024–1032.
8. Schlepütz M, Rieg AD, Seehase S, Spillner J, Perez-Bouza A, Braunschweig T, *et al.* Neurally mediated airway constriction in human and other species: a comparative study using precision-cut lung slices (PCLS). *PLoS One* 2012;7:e47344.
9. Neuhaus V, Danov O, Konzok S, Obernolte H, Dehmel S, Braubach P, *et al.* Assessment of the cytotoxic and immunomodulatory effects of substances in human precision-cut lung slices. *J Vis Exp* 2018;135: e57042.
10. Wronski S, Beinke S, Obernolte H, Belyaev NN, Saunders KA, Lennon MG, *et al.*; U-BIOPRED Study Group. Rhinovirus-induced human lung tissue responses mimic chronic obstructive pulmonary disease and asthma gene signatures. *Am J Respir Cell Mol Biol* 2021;65:544–554.
11. Bartlett NW, Slater L, Glanville N, Haas JJ, Caramori G, Casolari P, *et al.* Defining critical roles for NF-κB p65 and type I interferon in innate immunity to rhinovirus. *EMBO Mol Med* 2012;4: 1244–1260.
12. Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, *et al.*; U-BIOPRED Project Team. A transcriptome-driven analysis of epithelial brushings and bronchial biopsies to define asthma phenotypes in U-BIOPRED. *Am J Respir Crit Care Med* 2017;195: 443–455.
13. Steiling K, van den Berge M, Hijazi K, Florido R, Campbell J, Liu G, *et al.* A dynamic bronchial airway gene expression signature of chronic obstructive pulmonary disease and lung function impairment. *Am J Respir Crit Care Med* 2013;187:933–942.
14. Danov O, Lasswitz L, Obernolte H, Hesse C, Braun A, Wronski S, *et al.* Rupintrivir reduces RV-induced T$_H$-2 cytokine IL-4 in precision-cut lung slices (PCLS) of HDM-sensitized mice ex vivo. *Respir Res* 2019; 20:228.
15. Goris K, Uhlenbruck S, Schwegmann-Wessels C, Köhl W, Niedorf F, Stern M, *et al.* Differential sensitivity of differentiated epithelial cells to respiratory viruses reveals different viral strategies of host infection. *J Virol* 2009;83:1962–1968.
16. Dinnon KH III, Leist SR, Schäfer A, Edwards CE, Martinez DR, Montgomery SA, *et al.* A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures. *Nature* 2020;586:560–566.