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SHORT COMMUNICATION

# Precision cut lung slices: an ex vivo model for assessing the impact of immunomodulatory therapeutics on lung immune responses

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## Abstract

Chronic inflammatory diseases of the respiratory tract, such as chronic obstructive pulmonary disease (COPD) and asthma, are severe lung diseases that require effective treatments. In search for new medicines for these diseases, there is an unmet need for predictive and translatable disease-relevant in vitro/ex vivo models to determine the safety and efficacy of novel drug candidates. Here, we report the use of precision cut lung slices (PCLS) as a potential ex vivo platform to study compound effects in a physiologically relevant environment. PCLS derived from an elastase-challenged mouse model display key characteristics of increased inflammation ex vivo, which is exacerbated further upon challenge with LPS, mimicking the immune insult of a pathogen triggering disease exacerbation. Such LPS-induced inflammatory conditions are significantly abrogated by immunomodulatory agents targeting specific inflammatory signaling pathways in the absence of cytotoxic effects in lung slices. Thus, an ex vivo model of PCLS with a simulated pathogenic insult can replicate proposed in vivo pharmacological effects and thus could potentially act as a valuable tool to investigate the underlying mechanisms associated with lung safety, therapeutic efficacy and exacerbations with infection.

**Keywords** Lung inflammation · Ex vivo models · Precision cut lung slices · Immunomodulatory therapeutics

## Introduction

Patients diagnosed with chronic obstructive pulmonary disease (COPD) and asthma often experience acute exacerbations upon exposure to infection, leading to persistent pulmonary inflammation, detrimental lung destruction, and respiratory failure (Collaborators 2017; Sears 2008; Viniol and Vogelmeier 2018). Although such persistent lung inflammation can be alleviated by immunomodulatory drugs in the clinic (Camargo et al. 2009; Singh, 2019), a key question remains over the impact of anti-inflammatory therapies on host defense, particularly whether these treatments may reduce the inflammatory environment and thus potential exacerbation of subsequent lung damage. This highlights the need for translatable disease-relevant in vitro/ex vivo models to determine the efficacy and safety of novel drug candidates.

Airway instillation of porcine pancreatic elastase (PPE) has been documented to replicate the disease pathologies of emphysema and COPD in laboratory animals (Antunes and Rocco 2011). PPE instillation induced increased airway inflammation, goblet cell hyperplasia/metaplasia, airway

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mucus accumulation, and multifocal loss of tissue structure in mouse lungs, similar to disease pathologies noted in human COPD (Fernandez-Blanco, 2018). However, the utility of such models in safety and efficacy assessment has not been fully explored.

Precision cut lung slices (PCLS) provide the unique advantage compared to single cell or co-culture in vitro systems for modeling lung inflammatory diseases, as they correlate cell-specific functions with organ physiology and meet the principles of the Replacement, Refinement, Reduction of Animals in research. PCLS from healthy or smoke-exposed mice replicate responses of human donor PCLS, e.g., excessive inflammation and airway contraction, upon exposure to Influenza A infection and cigarette smoke (Bauer, 2010; Donovan et al. 2016; Temann et al. 2017). Here, we propose PCLS can be used as a valuable ex vivo model for assessing the impact of immunomodulatory therapies on infection-induced inflammatory exacerbation in the PPE-challenged mouse lung. We have utilized lipopolysaccharide (LPS) to replicate a pathogen insult and defined differences in the immune response noted between healthy and 'diseased' lung slice cultures in the presence of inhibitors targeting immune pathways associated with host defense. The PCLS model has the potential to provide an in-depth understanding of molecular mechanisms underlying respiratory disease alongside the investigation of the efficacy and safety of inhaled therapeutics.

## Materials and methods

### Mouse model and animal ethics

Female 20-week C57BL/6 N mice were purchased from Charles River (Sulzfeld, Germany) and maintained at the animal facility under specific pathogen-free conditions with food and water ad libitum. Mice were handled following standards established by the Council of Europe, the Declaration of Helsinki, Swedish legislation, and AstraZeneca Global Internal Standards. All experiments were performed under an ethical permit approved by the Gothenburg Ethics Committee for Experimental Animals in Sweden.

Mice were put under light isoflurane anesthesia before exposed to 0.6 units of PPE/saline (3,015,959, EMD Millipore) or 50 µL saline intranasally at day 0 and day 7, and terminated at day 14 with an intraperitoneal injection of 0.2 mL pentobarbital sodium (Apoteksbolaget, Sweden). Six mice were allocated per treatment group to gain the power of statistical significance (Percie du Sert, 2020).

### Histopathological analysis

Mouse lungs ($n = 3$ per treatment group) were perfused through the interventricular septum with PBS and then inflated with 4% paraformaldehyde by intratracheal instillation. The trachea was tied and the inflated lungs were removed and immediately immersed in 4% paraformaldehyde. After being dehydrated in ethanol and xylene and embedded in paraffin, the lungs were sectioned at 5 µm and subjected to hematoxylin and eosin (H&E) staining for qualitative evaluation of inflammation and structural alterations. Alcian blue/periodic acid-Schiff (AB-PAS) staining was performed for qualitative assessment of goblet cell hyperplasia.

### Flow cytometric analysis of immune cells in mouse lungs

Mouse lungs were inflated via the trachea with 2 ml digestion solution containing 2 mg/mL Collagenase/Dispase (10,269,638,001, Roche) in HBSS plus 5% fetal bovine serum and 10 mM HEPES. The trachea was tied and immediately immersed in ice-cold PBS. The lungs were chopped into small pieces and put into gentleMACS C tubes containing 5 mL digestion solution. The lung pieces were then minced for 5 min with a gentleMACS dissociator (Miltenyi Biotec), and incubated with 0.5 mg/mL DNase I (10,104,159,001, Roche) at 37°C for 45 min with gentle vortexing every 10 min. Upon completion of digestion, the resulting cell suspensions were strained through a 100-µm cell strainer and treated with ACK lysing buffer (A10492, Gibco). After counting, cells at $1 \times 10^6$ per sample were stained with 0.2 mL Aqua Live/Dead viability dye (L34957, Life Technologies) at room temperature in the dark for 30 min, followed by incubation on ice for 30 min in 0.2 mL blocking solution containing 5% normal mouse serum (M5905, Sigma-Aldrich), 5% normal rat serum (R9759, Sigma-Aldrich), and 1% FcBlock (553,141, BD Biosciences). The cells were then stained with Brilliant Violet 605 anti-mouse CD45 antibody (563,053, BD Bioscience) at room temperature for 30 min. After staining, cells were washed and fixed with 4% paraformaldehyde in PBS. Data were acquired with a BD LSRFortessa cell analyzer using BD FACSDiva software (BD Bioscience) and analyzed using Flowjo v10.

### Precision cut lung slices (PCLS)

Mouse lungs were inflated via the trachea with a solution of 1.5% low melting point agarose (16,520,050, Invitrogen) in PBS at 37-40°C. After removal, the lungs were immediately immersed in cold PBS placed on ice for 30 min to solidify

the agarose. The lung lobes were punctured to produce cylindrical cores using a 6-mm tissue coring tool and PCLS were generated through cutting the cores at 300-µm thickness by the Krumdieck slicer (MD2000, Alabama Research & Development) filled with cold PBS. After cutting, lung slices from the same treatment group (approximately 130 slices) were collected in one petri dish to obtain a random mix of tissues originating from all 6 animals per treatment group and covering different parts of the lung including and between the upper and lower lobes. The culture medium used during this step was SAGM (Small Airway Epithelial Cell Growth Media, Lonza, CC-3118) supplemented with gentamycin/amphotericin B to prevent pathogen contamination from the onset of culture. PCLS were washed twice with SAGM before overnight incubation at 37°C, 5% $CO_2$, and 95% humidity to allow slices to acclimatize prior experiments. The following day, a single slice per well was submerged in 0.15 mL SAGM without supplements in 96-well cell culture plates and consequently subjected to compound treatment (see Supplemental Fig. 1).

### Compound treatment and PCLS assays

The compounds assessed in this study include Interleukin-1 receptor antagonist (IL-1Ra) Anakinra (kineret®, Sobi), Phosphoinositide 3-kinase delta (PI3Kδ) inhibitor Idelalisib (ZYDELIG®, GILEAD), and Janus kinase (JAK) inhibitor Tofacitinib (XELJANZ®, Pfizer). Lipopolysaccharide (LPS) from *Escherichia coli* O26: B6 was purchased from Sigma-Aldrich (L8274). The assay media consisted of SAGM medium without antibiotics, and 96-well plates were used as assay format.

Before commencing LPS and compound treatments, PCLS were washed with warm SAGM. To determine whether PCLS respond differently to LPS treatment, lung slices from both naive and PPE-challenged mice were treated with 0.5, 5, and 10 ng/mL LPS for 24 h ($n = 3$ slices). To determine the impact of compound exposure on LPS challenge, PCLS were pre-incubated with compounds at 1, 5, and 10 µM concentrations for 2 h, followed by 24 h LPS treatment ($n = 3$ for naïve, $n = 3$ or 5 for PPE). LPS concentrations were applied at 0.5 ng/mL for Anakinra and 5 ng/mL for Idelalisib and Tofacitinib, following optimization. Culture supernatants were subjected to cytokine and cytotoxicity assays and stored at −20 °C. PBS was used as vehicle control (veh) for Anakinra, and 0.1% DMSO as vehicle control for Idelalisib and Tofacitinib.

### Cytokine analysis

Cytokine levels in the culture supernatants were measured by Luminex multiplex bead assays. Specifically, ProcartaPlex multiplex Luminex immunoassays (Invitrogen) were used to quantify mouse interleukin 6 (IL-6), monocyte chemoattractant protein 1 (MCP-1), and keratinocytes-derived chemokine (KC) (also known as C-X-C motif chemokine receptor 1, CXCL1, in human), according to the manufacturer's instructions.

### Cytotoxicity and viability assays

Cytotoxicity was evaluated by measuring lactate dehydrogenase (LDH) release in culture supernatants by LDH-Glo™ Cytotoxicity Assay Kit (J2381, Promega) according to the manufacturer's instructions. PCLS were treated with 1% Triton X-100 as positive controls of cell death.

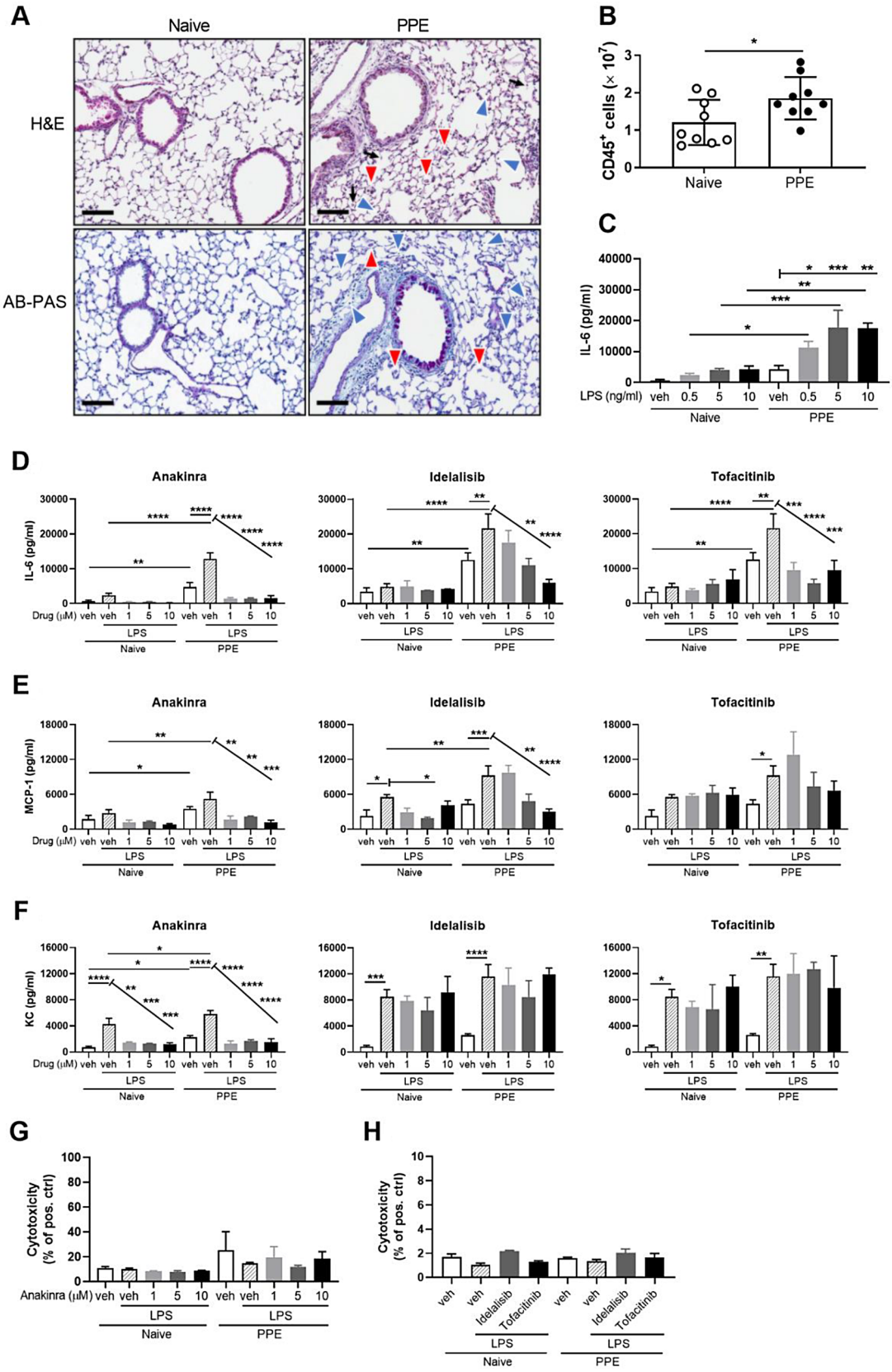
### Statistical analysis

The difference between experimental results was analyzed using GraphPad Prism v8.0 with a $p$ value of $< 0.05$ considered to be statistically significant. The overall $p$ values for analyses in the figures were all $< 0.05$, and the $p$ values reported in the figure legend represent the post hoc tests. Data were analyzed using a two-tailed unpaired $t$ test with Welch's correction or a one-way ANOVA multiple comparisons, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

## Results

### PPE challenge induces minimal alveolar inflammation and airway remodeling in the mouse lung

Upon intranasal exposure of PPE or saline over 14 days, PPE-challenged mice showed a slight increase in infiltrated immune cells around the intrapulmonary airways and alveoli, mainly macrophages and mononuclear inflammatory cells, whereas no signs of inflammation were observed in the lungs of saline-treated naïve mice (Fig. 1A). Further, PPE-challenged mice displayed an increase of AB/PAS-positive cells in the bronchial epithelium, demonstrating goblet cell hyperplasia/metaplasia induced by PPE (Fig. 1A). These alterations were accompanied by a significant increase in infiltrating CD45-positive immune cells in PPE-challenged mice, determined by flow cytometry (Fig. 1B). In summary, airway instillation of PPE induces alveolar inflammation and airway remodeling in mice, which recapitulates key characteristics of human COPD and thus has the potential to provide lung tissue with an underlying disease context for ex vivo models.


A
Naive
PPE
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AB-PAS
B
CD45+ cells (× 10^7)
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IL-6 (pg/ml)
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◂**Fig. 1** Application of PCLS as an ex vivo model for assessing the impact of immunomodulatory therapeutics on lung immune responses. Mice were exposed to either PPE or saline naïve control, and lung sections were analyzed histologically thereafter. **A** H&E stain revealed infiltration of immune cells around the intrapulmonary airways and alveoli in PPE-challenged mice (upper right panel, red arrowheads: macrophages; blue arrowheads: small mononuclear cells; black arrows: eosinophilic debris). AB-PAS stain showed goblet cell hyperplasia/metaplasia in the airways in PPE-challenged mice (lower right panel, **A**). Scale bar, 100 µm. **B**. Increased infiltration of CD45-positive immune cells was evaluated by flow cytometry in the lung of PPE-challenged mice. Two-tailed unpaired t test with Welch's correction was used for **B**, $n=9$ per group. **C** The inflammation of PCLS from PPE-challenged mice was exacerbated by LPS treatment, indicated as a dose-dependent and significant enhancement of IL-6 secretion compared to naïve lung slices. Anakinra significantly inhibit LPS-induced enhanced secretion of IL-6, MCP-1, and KC in the PPE PCLS (**D**, **E**, **F**). Idelalisib significantly inhibits LPS-induced enhanced secretion of IL-6 and MCP-1 in the PPE PCLS in a dose-dependent manner, while Tofacitinib significantly and dose-dependently inhibits LPS-induced enhanced secretion of IL-6 alone in the PPE PCLS (**D**, **E**, **F**). LPS at 0.5 ng/mL was used for Anakinra and 5 ng/mL for Idelalisib and Tofacitinib. Furthermore, LDH cytotoxicity assays reveal that all drugs have no cytotoxic effects on lung slices in the presence and absence of LPS, while positive controls demonstrate full cytotoxicity (**G**, **H**). In **H**, Idelalisib and Tofacitinib at 10 µM are shown. Data in figures **C-H** are shown as mean ± SEM and analyzed using a one-way ANOVA multiple comparisons, $*p<0.05$, $**p<0.01$, $***p<0.001$, and $****p<0.0001$ (color figure online)

### PCLS from PPE-challenged mice show elevated inflammation upon LPS treatment

To replicate the inflammatory exacerbations seen in patients with pathogen infection, lung slices from naïve and PPE-challenged mice were treated with LPS. In line with the increased infiltration of immune cells in the airways in vivo (Fig. 1B), PCLS from PPE-challenged mice showed baseline levels of IL-6 release 6.5-fold higher compared with lung slices from naïve mice ex vivo (Fig. 1C). This inflammation was further significantly enhanced upon LPS treatment in a dose-dependent manner (Fig. 1C). These findings suggest that PCLS as an ex vivo model can capture key aspects of baseline disease inflammation and exacerbation in response to a simulated pathogenic insult.

### Immunomodulatory drugs significantly modulate LPS-induced inflammation in both PCLS from naïve and PPE-challenged mice

To determine how immunomodulatory compounds impact on LPS-induced exacerbation of inflammation, PCLS were incubated with Anakinra (IL-1R antagonist), Idelalisib (PI3Kδ inhibitor), and Tofacitinib (JAK inhibitor), which are known to suppress specific inflammatory signaling pathways and increase infection risk in the clinic (Alflen et al. 2018; Dinarello et al. 2012; Gao et al. 2016; Horak, 2016). Anakinra significantly inhibited LPS-induced secretion of IL-6, MCP-1, and KC in PCLS from both naïve and PPE-challenged mice (Fig. 1D-F). Similarly, Idelalisib inhibited the LPS-enhanced secretion of IL-6 and MCP-1 while Tofacitinib only significantly inhibited IL-6 release in the PPE PCLS (Fig. 1D-F). The inhibition of inflammatory mediators was accompanied by no detectable cytotoxic effects of all tested drugs on lung slices compared to cytotoxicity positive control (Fig. 1G-H). In summary, this ex vivo model of PPE PCLS using tissue from an inflamed lung permitted detection of significant immunological changes following treatment with immunomodulatory compounds in vitro.

## Discussion

Prediction of potential adverse inflammation and infection risk is a key challenge given current in vivo toxicology assessments utilize clean animal facilities and healthy, young animals. Application of lung slices generated from in vivo disease models is particularly valuable for modeling lung inflammatory diseases because of the unique advantage of PCLS with its intact tissue architecture. Here, we have demonstrated that lung slices from PPE-challenged mice reproduce key features of the inflammatory exacerbations noted clinically upon infection in the inflammatory lung disease population (e.g., COPD and asthma). Through mimicking these acute exacerbations, the PCLS ex vivo model can potentially be used to investigate efficacy alongside safety concerns such as increased infection risk.

Access to translational models to assess the impact of drug candidates is of key importance for successful Drug Discovery and Development. In a respiratory disease context, suppressing lung inflammation through inhibition of specific immune pathways is of clinical benefit to improve symptoms and reduce exacerbation risk of COPD and asthma. IL-1R, PI3K, and JAK are signaling molecules that modulate various key mechanisms in the inflammatory response to external insults, such as LPS, leading to distinct cytokine profiles (Calama et al. 2013; Guha and Mackman 2002; Kallapur et al. 2009). Therefore, these signaling molecules represent new potential drug targets in respiratory inflammatory diseases, as they have been demonstrated to be activated and correlate with disease severity and exacerbations of COPD and asthma (Baines et al. 2017; Evans et al. 2018; Marwick, 2010; Southworth, 2018; Yew-Booth et al. 2015). Preclinical and clinical studies have also confirmed that inhibition of these pathways has the potential to attenuate detrimental inflammation, improve lung function, and/or lower exacerbation risk in the context of chronic inflammation or COPD and asthma in vivo studies (Park, 2010; Southworth, 2016; To, 2010). However, targeting these pathways also increases the risk of severe infection, as noted in the clinical data for Anakinra, Idelalisib, and Tofacitinib, inhibitors of the IL-1R, PI3K and JAK pathways, respectively

(Fleischmann, 2006; Hawkins and Stephens 2015; Mantovani et al. 2019; Villarino et al. 2015).

In this study, we used LPS to mimic the immune insult of a pathogen triggering disease exacerbation. The compounds tested (Anakinra, Idelalisib and Tofacitinib) ameliorate LPS-induced inflammatory signaling over the pre-existing inflammatory state of lung slices taken from a PPE in vivo model. Inhibition of the early, innate immune pathways (through IL-1 inhibition) had a more pronounced impact on the release of the cytokines descriptive of early responses. Inhibition of the cytokine signaling pathways through PI3K and JAK afforded inhibition of IL-6 and MCP-1, but not KC. This indicates that the lung slice model may be informative for interrogating alternative signaling cascades with more in-depth cytokine profiling (secretomic) or transcript profiling end points. This preliminary investigation demonstrates that inhibition of responses induced by pathogen-associated molecular patterns (PAMPs) such as LPS can be detected and provides a baseline for live pathogen assessment in subsequent studies. Thus, the PCLS model represents a valuable ex vivo model for preclinical assessment of immunomodulation in inflammatory respiratory diseases.

In conclusion, PCLS have a fundamental advantage over in vitro models given the resident immune component, intact architecture, and potential to be derived from disease models where the immune context is altered and of increasing importance for toxicity testing. Although PCLS lack the critical and recruitable immune components, this system is of potential benefit in the assessment of innate immune responses and adverse immunosuppressive risks such as host defense, particularly when working alongside other complex human cell culture systems, such as Air Liquid Interface (Balogh Sivars, 2018) and human PCLS. The build of this mouse PCLS model as an immune-competent ex vivo system with which to interrogate adverse outcomes of immune modulation is continuing with a lung-relevant pathogen overlay alongside exposure to novel drug candidates.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00204-021-03096-y.

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## Declarations

**Conflict of interest** Guanghui Liu, Linnea Särén, Helena Douglasson, Xiao-Hong Zhou, Per M Åberg, Anna Ollerstam, Catherine J Betts and Kinga Balogh Sivars are employees of AztraZeneca.

## References

Alflen A, Stadler N, Aranda Lopez P, Teschner D, Theobald M, Hess G, Radsak MP (2018) Idelalisib impairs TREM-1 mediated neutrophil inflammatory responses. Sci Rep 8:5558. https://doi.org/10.1038/s41598-018-23808-2

Antunes MA, Rocco PR (2011) Elastase-induced pulmonary emphysema: insights from experimental models. An Acad Bras Cienc 83:1385–1396. https://doi.org/10.1590/s0001-37652011005000039

Baines KJ, Fu JJ, McDonald VM, Gibson PG (2017) Airway gene expression of IL-1 pathway mediators predicts exacerbation risk in obstructive airway disease. Int J Chron Obstruct Pulmon Dis 12:541–550. https://doi.org/10.2147/COPD.S119443

Balogh Sivars K et al (2018) A 3D human airway model enables prediction of respiratory toxicity of inhaled drugs. Vitro Toxicol Sci 162:301–308. https://doi.org/10.1093/toxsci/kfx255

Bauer CM et al (2010) Treating viral exacerbations of chronic obstructive pulmonary disease: insights from a mouse model of cigarette smoke and H1N1 influenza infection. PLoS ONE 5:e13251. https://doi.org/10.1371/journal.pone.0013251

Calama E, Domenech A, Carreño C, De Alba J, Prats N, Miralpeix M, Ramis I (2013) Involvement of the JAK-STAT3 pathway in a lipopolysaccharide-induced rat model of airway inflammation. Eur Respir J 42(Suppl. 57):733

Camargo CA Jr, Rachelefsky G, Schatz M (2009) Managing asthma exacerbations in the emergency department: summary of the national asthma education and prevention program expert panel report 3 guidelines for the management of asthma exacerbations. J Allergy Clin Immunol 124:S5–S14. https://doi.org/10.1016/j.jaci.2009.05.010

Collaborators GBDCRD (2017) Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Respir Med 5:691–706. https://doi.org/10.1016/S2213-2600(17)30293-X

Dinarello CA, Simon A, van der Meer JW (2012) Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat Rev Drug Discov 11:633–652. https://doi.org/10.1038/nrd3800

Donovan C, Seow HJ, Bourke JE, Vlahos R (2016) Influenza A virus infection and cigarette smoke impair bronchodilator responsiveness to beta-adrenoceptor agonists in mouse lung. Clin Sci (lond) 130:829–837. https://doi.org/10.1042/CS20160093

Evans MD, Esnault S, Denlinger LC, Jarjour NN (2018) Sputum cell IL-1 receptor expression level is a marker of airway neutrophilia and airflow obstruction in asthmatic patients. J Allergy Clin Immunol 142:415–423. https://doi.org/10.1016/j.jaci.2017.09.035

Fernandez-Blanco JA et al (2018) Attached stratified mucus separates bacteria from the epithelial cells in COPD lungs. JCI Insight. https://doi.org/10.1172/jci.insight.120994

Fleischmann RM et al (2006) Safety of extended treatment with anakinra in patients with rheumatoid arthritis. Ann Rheum Dis 65:1006–1012. https://doi.org/10.1136/ard.2005.048371

Gao W, McGarry T, Orr C, McCormick J, Veale DJ, Fearon U (2016) Tofacitinib regulates synovial inflammation in psoriatic arthritis, inhibiting STAT activation and induction of negative feedback inhibitors. Ann Rheum Dis 75:311–315. https://doi.org/10.1136/annrheumdis-2014-207201

Guha M, Mackman N (2002) The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. J Biol Chem 277:32124–32132

Hawkins PT, Stephens LR (2015) PI3K signalling in inflammation. Biochim Biophys Acta 1851:882–897. https://doi.org/10.1016/j.bbalip.2014.12.006

Horak F et al (2016) Randomized phase 1 study of the phosphatidylinositol 3-kinase delta inhibitor idelalisib in patients with allergic rhinitis. J Allergy Clin Immunol 137:1733–1741. https://doi.org/10.1016/j.jaci.2015.12.1313

Kallapur SG, Nitsos I, Moss TJ et al (2009) IL-1 mediates pulmonary and systemic inflammatory responses to chorioamnionitis induced by lipopolysaccharide. Am J Respir Crit Care Med 179:955–961

Mantovani A, Dinarello CA, Molgora M, Garlanda C (2019) Interleukin-1 and related cytokines in the regulation of inflammation and immunity. Immunity 50:778–795. https://doi.org/10.1016/j.immuni.2019.03.012

Marwick JA et al (2010) A role for phosphoinositol 3-kinase delta in the impairment of glucocorticoid responsiveness in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 125:1146–1153. https://doi.org/10.1016/j.jaci.2010.02.003

Park SJ et al (2010) Phosphoinositide 3-kinase delta inhibitor suppresses interleukin-17 expression in a murine asthma model. Eur Respir J 36:1448–1459. https://doi.org/10.1183/09031936.00106609

Percie du Sert N et al (2020) The ARRIVE guidelines 2.0 Updated guidelines for reporting animal research. PLoS Biol 18:3000410. https://doi.org/10.1371/journal.pbio.3000410

Sears MR (2008) Epidemiology of asthma exacerbations. J Allergy Clin Immunol 122:662–668. https://doi.org/10.1016/j.jaci.2008.08.003

Singh D et al (2019) Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease: the GOLD science committee report 2019. Eur Respir J. https://doi.org/10.1183/13993003.00164-2019

Southworth T et al (2016) Anti-inflammatory potential of PI3Kdelta and JAK inhibitors in asthma patients. Respir Res 17:124. https://doi.org/10.1186/s12931-016-0436-2

Southworth T et al (2018) PI3K, p38 and JAK/STAT signalling in bronchial tissue from patients with asthma following allergen challenge. Biomark Res 6:14. https://doi.org/10.1186/s40364-018-0128-9

Temann A, Golovina T, Neuhaus V, Thompson C, Chichester JA, Braun A, Yusibov V (2017) Evaluation of inflammatory and immune responses in long-term cultured human precision-cut lung slices. Hum Vaccin Immunother 13:351–358. https://doi.org/10.1080/21645515.2017.1264794

To Y et al (2010) Targeting phosphoinositide-3-kinase-delta with theophylline reverses corticosteroid insensitivity in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 182:897–904. https://doi.org/10.1164/rccm.200906-0937OC

Villarino AV, Kanno Y, Ferdinand JR, O'Shea JJ (2015) Mechanisms of Jak/STAT signaling in immunity and disease. J Immunol 194:21–27. https://doi.org/10.4049/jimmunol.1401867

Viniol C, Vogelmeier CF (2018) Exacerbations of COPD. Eur Respir Rev. https://doi.org/10.1183/16000617.0103-2017

Yew-Booth L, Birrell MA, Lau MS, Baker K, Jones V, Kilty I, Belvisi MG (2015) JAK-STAT pathway activation in COPD. Eur Respir J 46:843–845. https://doi.org/10.1183/09031936.00228414