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Single-cell mapping reveals CXCL11 as a driver of mucus production and inflammation in influenza A virus exacerbation in COPD

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ABSTRACT

Influenza A virus (IAV)–induced exacerbations are a major contributor to morbidity in chronic obstructive pulmonary disease (COPD), yet the epithelial mechanisms that govern these events remain unknown. We profiled the response to IAV infection of differentiated airway epithelial cells from healthy donors and individuals with COPD at single-cell resolution. The analysis revealed infection-driven shifts across multiple epithelial compartments and distinct alterations in cell–cell communication in COPD, associated with an increased CXCL11 expression. Functional assays demonstrated that CXCL11 augments mucus-associated gene and protein expression, particularly MUC5AC, increases mucus secretion and viscosity and is associated with reduction of virus-related immune pathways. This highlights CXCL11 as a contributor to both mucus hypersecretion and impaired antiviral epithelial responses in COPD exacerbations.

Chronic obstructive pulmonary disease (COPD), the third leading global cause of death, is marked by irreversible airflow limitation and epithelial remodelling.[1] Exacerbations drive morbidity and mortality, with influenza A virus (IAV) being a major trigger. The airway epithelium is both the primary infection site and the initiator of immune responses.[2] Evidence suggests that the COPD epithelium has a delayed antiviral response contributing to worse outcomes,[3] but the underlying molecular pathways and cellular drivers remain unclear. To address this, we generated single-cell transcriptomics data (figure 1A) from primary bronchial epithelial cells (BEC) from healthy donors and COPD patients, without any known genetic predisposition (table 1), cultured in an air-liquid interface (ALI) and exposed to IAV infection. Bronchial brush–derived cells were cultured submerged to confluency and then air-lifted for 28 days to maturation before IAV infection (MOI=1, 24 hours, strain A/Hamburg/5/09). Analysis of viral transcripts confirmed successful infection with no group differences (data not shown). Single-cell libraries were generated using BD Rhapsody, sequenced on the Illumina NovaSeq 6000 S4, and 97 573 cells were annotated via scANVI-scArches with the Human Lung Cell Atlas[4] as reference (online supplemental file 1), identifying all major epithelial compartments including rare epithelial subtypes such as ionocytes[5 6] (figure 1B,C, online supplemental file 1 and figure 1). Previous studies without cell type resolution have shown differences in the response to IAV between healthy individuals and COPD patients;[7] therefore, we suspected that an altered cellular composition of the airway epithelium could explain these differences.

Indeed, cell type proportion analysis showed slight reduction of deuterosomal and ciliated cells and increased BPIFA1+Club/Goblet cells in COPD patients (figure 1D,E). Notably, stratifying samples based on infection status, independently of disease status, revealed significant enrichment of distinct cell states among basal, suprabasal, IFN-high Club/Goblet, ciliated and goblet cells. Moreover, we found enrichment of a small cluster co-expressing several epithelial markers but with a lacking lineage signature (undetermined cells) (figure 1F, Milopy, false discovery rate (FDR) <0.05). To examine how different cell types within the epithelium communicate during infection, we analysed potential ligand–receptor interactions (LIANA+) in ALIs from healthy individuals and patients with COPD after infection and assessed patterns of cell–cell communication separately in each group (Tensor-cell2cell).[8] We identified eight communication programmes activated by IAV infection, two of which were differentially regulated in COPD. The first programme was characterised by three main receiver cells (basal cells, suprabasal cells, and ionocytes) shown by their higher receiver loadings, associated with a down-regulation of the epidermal growth factor receptor (EGFR), mitogen-activated protein kinase (MAPK), tumour necrosis factor alpha (TNFα), and vascular endothelial growth factor (VEGF) signalling pathways in IAV-COPD samples (figure 1G, left panel). The second programme was characterised by two main senders (suprabasal and basal cells) driving the activation of the nuclear factor kappa B (NF-κB), Janus kinase/signal transducer and activator of transcription (JAK-STAT) and transforming growth factor-beta (TGF-β) signalling in the receiver cells in IAV-COPD samples (figure 1G, right panel).

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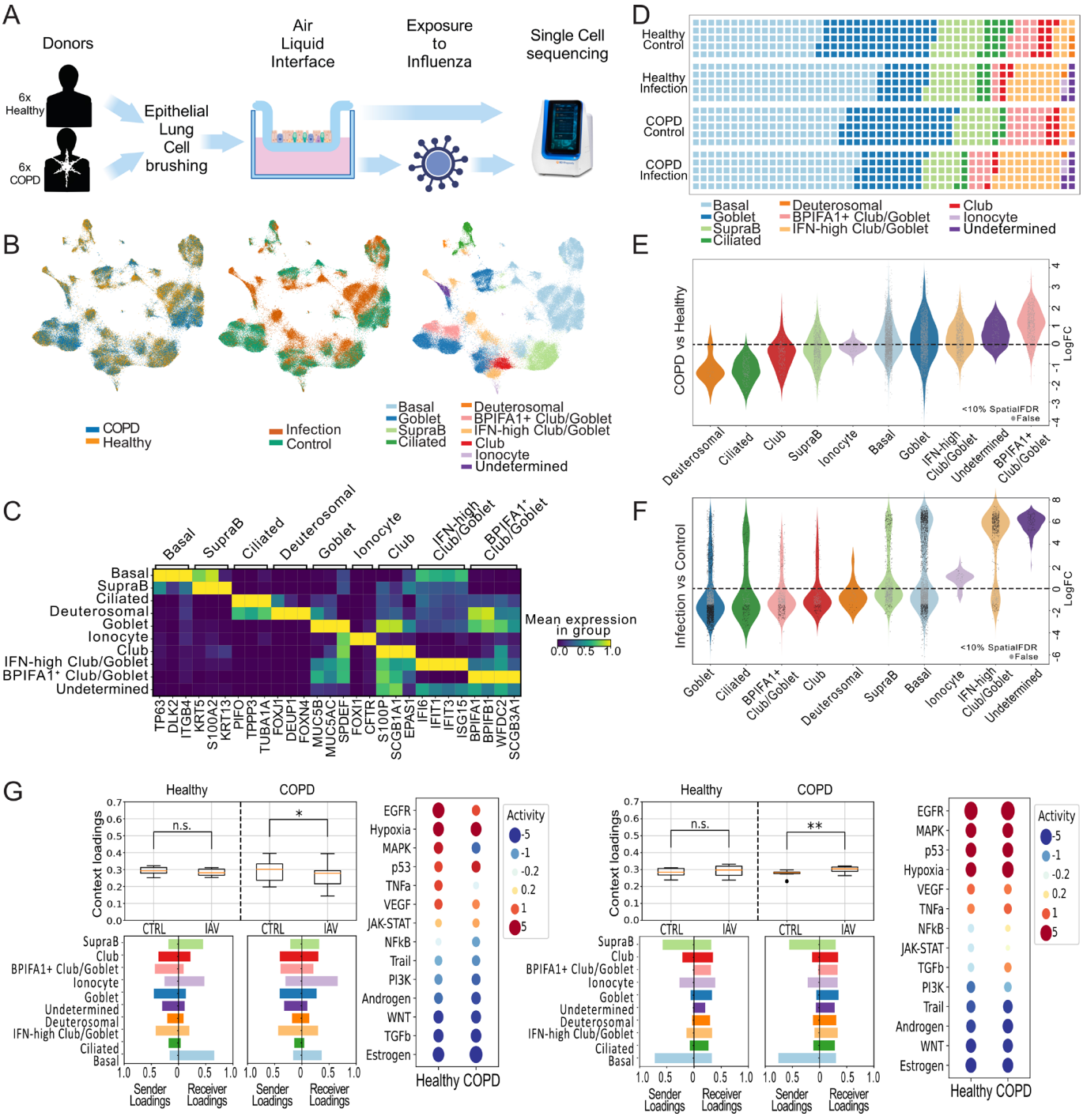

Figure 1 Pre-existing chronic obstructive pulmonary disease (COPD) alters airway epithelial composition and communication networks in response to influenza A virus (IAV). (A) Experimental design. Epithelial lung cells were isolated after lung cell brushing from healthy donors and COPD patients (n=6 biological replicates/group). Cells were cultured and differentiated in an air-liquid interface (ALI) model and exposed or not to IAV for 24 hours. At collection time, single-cell RNA analysis was performed by BD Rhapsody and Illumina NovaSeq6000 sequencing. (B) UMAP of clusters colour-coded by disease status, infection status and cell type. (C) Gene signatures of annotated cell types. The heatmap shows the average scaled expression of cell-type specific markers for each cell type. (D) Cell type proportion analysis of ALI cells from healthy/COPD patients exposed or not to IAV. Coloured squares indicate percentages of each cell type. (E) Fold change analysis of cell type proportions in ALI cells compared by disease independent of infection status. (F) Fold change analysis of cell type proportions in ALI cells compared by infection status independent of disease status. (G) Cell-cell communication factor analysis for healthy and COPD after IAV infection. Boxplots show context loadings of communication factors significantly deregulated in COPD versus Healthy (paired samples, CTRL and IAV). *p value<0.05, ** p value<0.01 after paired t-test with Benjamini–Hochberg correction. Bar plots display the main sender and receiver cell types contributing to each factor. Dot plots show the top associated signalling pathways in receiver cells for each factor. Dot colour and size represent the inferred activity.

Table 1 Patient characteristics, including demographic and clinical information by disease

Healthy donors		COPD donors	
Characteristic	N=6 *Mean (SD); n (%)*	Characteristic	N=6 *Mean (SD); n (%)*
Pack Years	7.50 (9.87)	Pack Years	39.17 (22.40)
FEV1 % predicted	111.78 (15.10)	FEV1 % predicted	73.13 (14.94)
Years of non-smoking	12.67 (12.22)	Years of non-smoking	12.20 (8.58)
Never-smoker	3	Never-smoker	1
Age	52.50 (10.13)	Age	67.83 (8.08)
BMI	28.10 (4.17)	BMI	28.96 (3.37)
Gender		Gender	
m	6 (100%)	m	6 (100%)

BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 second.

To identify relevant molecular mediators underlying these changes in cell-cell communication patterns after IAV infection, we performed a differential gene expression (DEG) analysis by cell type. Among the top 20 DEGs, we identified genes mainly associated with interferon-induced antiviral response (CMPK2, OASL, IFIT1-3, IFI44L, RSAD2, HERC5), innate immune sensing (ZBP1) and immune cell recruitment (CXCL10, CXCL11) (figure 2A). CXCL11 was the most significantly upregulated chemokine across the analysed cell types, suggesting an important role in the epithelial response to IAV infection (figure 2B). Notably, CXCL11 activates both NF-κB and JAK–STAT signalling pathways, both of which were shown to be altered in COPD-ALI after IAV infection (figure 1G) and it is elevated in the sputum of COPD patients.[9] Although CXCL11 is well known for its role in immune cell recruitment, its impact on the airway epithelium is not well understood. Prior studies demonstrated that airway epithelial cells express CXCL11 receptors (CXCR3, CXCR7/ACKR3),[9–11] and in our dataset, we found that CXCR7/ACKR3 is upregulated in COPD and further induced after IAV infection (figure 2C). Therefore, we proceeded to investigate the direct effects of CXCL11 on the airway epithelium at baseline and after IAV infection. To this end, we generated ALI cultures from healthy donor BEC, exposed them to CXCL11 (low, 10 ng/mL; high, 50 ng/mL) for 28 days and performed bulk-RNA sequencing (Novogene, reference genome Hg38) (figure 2D). Here, we found a distinct phenotype induced by CXCL11 (figure 2E), in which the expression of several genes involved in mucus production and secretion,

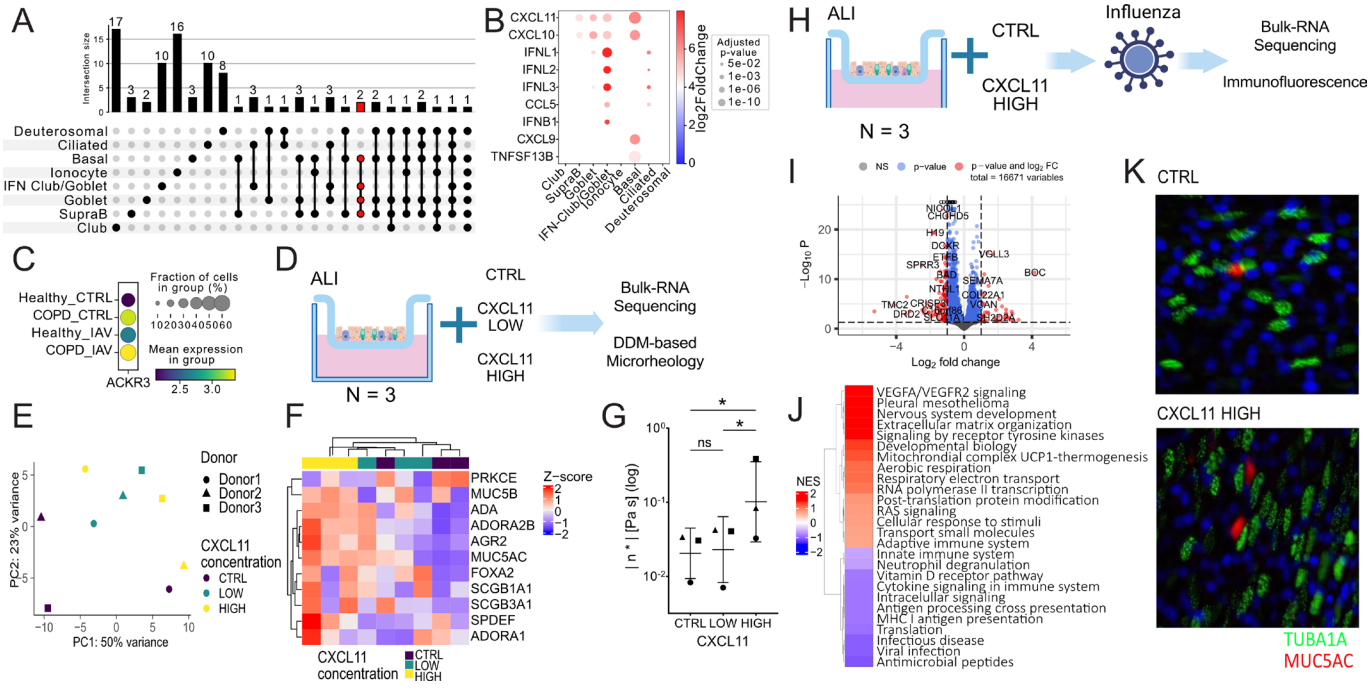

Figure 2 CXCL11 alters mucus secretion and immune response to IAV in an ALI model. (A) Upset plot of top 20 differentially expressed genes (DEG) among cell types after IAV infection. Connected lines represent cell types with shared DEG shown by bars. (B) Bubble plot of top upregulated chemokines/cytokines in the DEG per cell type (Log2FoldChange: IAV vs CTRL). Dot colour shows log2FC and dot size adjusted p-values. (C) Bubble plot showing mean gene expression of the CXCL11 receptor ACKR3 (colour) and fraction of expressing cells (size) by disease and infection status. (D) Experimental design for mucus viscosity measurements and bulk RNA sequencing of healthy ALI culture exposed to different concentrations of CXCL11. (E) PCA plot after bulk RNA sequencing. Points with different shapes indicate biological replicates (n=3). Different colours show CXCL11 concentration (CTRL, LOW, HIGH). (F) Normalised gene expression of genes associated with mucus secretion in ALI exposed to different CXCL11 concentrations. (G) Mucus complex viscosity measured by differential dynamic microscopy (DDM)–based microrheology[15 16] in supernatants from healthy ALI exposed to different CXCL11 concentrations. Data represent the log10-transformed mean viscosity per biological replicate (n=3). *p value <0.05, ns: non-significant; after repeated-measures analysis of variance followed by Tukey's multiple comparisons test. (H) Experimental design for bulk RNA sequencing and immunofluorescence of ALI culture exposed to IAV infection in absence (CTRL) or presence of CXCL11 (HIGH). (I) The volcano plot shows DEG induced by CXCL11 in infected ALI cultures. (J) Pathway enrichment analysis based on DEG (LFC>1, padj<0.05). (K) Representative images of immunostaining for mucin 5AC (MUC5AC) and tubulin alpha 1A (TUBA1A) in infected ALI cultures exposed to different CXCL11 concentrations (n=4, biological replicates). ALI, air-liquid interface; IAV, influenza A virus.

including MUC5AC, MUC5B, and SPDEF, was strongly upregulated in a dose-dependent manner (figure 2F). Additionally, CXCL11-treated ALI cultures produced significantly more viscous mucus (figure 2G, online supplemental file 1 and figure 2). Next, we explored if CXCL11 altered the response to IAV (figure 2H). Bulk RNA sequencing data showed various differentially expressed genes (figure 2I) driving the downregulation of immune-associated pathways in comparison to non-CXCL11 infected ALI cultures, suggesting an impaired response to IAV infection (figure 2J). Moreover, CXCL11 further upregulated the expression of mucins such as MUC5AC in infected ALI cultures (figure 2K).

In this report, we investigated molecular mechanisms underlying the response of the lung epithelium to IAV infection using ALI cultures. The cellular communication networks induced by infection differed between healthy and COPD airway cells, with pathways including NF-κB and JAK-STAT being differentially regulated. Prior studies showed that CXCL11 is induced after IAV infection,[9] which we could confirm. Our study adds cell type resolution to this finding and shows that CXCL11 was specifically upregulated in basal, suprabasal, club and goblet cells. Previously, CXCL11 has been associated with T cell recruitment.[9] Here, we highlight its role in epithelial function and response to infection. Exogenous CXCL11 was associated with heightened mucus production at baseline and an impaired immune response on infection.

COPD exacerbations are positively associated with increased mucin production and altered clearance at the onset of the exacerbation, a process to which, based on our data, CXCL11 could contribute.[12] While the exact mechanism by which CXCL11 induces mucin production in specific epithelial cell types remains unclear, evidence suggests that it may involve NF-κB signalling in lung epithelial cells, a pathway showing COPD-specific regulation in our analysis.[13] NF-κB can function either upstream or downstream of CXCL11, depending on the context, and it also interacts with interferon signalling. Both the NF-κB and interferon pathways can induce CXCL11 expression, and CXCL11 in turn may help reinforce these pathways through feedback loops.[14] Disentangling these complex signalling associations will require further studies, eventually contributing to identifying therapeutic targets for COPD exacerbation.

This study has several limitations that should be considered when interpreting the findings. The COPD donors were older on average than the healthy controls, and age-related alterations in antiviral responsiveness cannot be fully excluded as contributing factors. Similarly, although none of the donors were current smokers, total long-term smoking exposure differed between groups, and the study was not powered to disentangle effects of cumulative smoke exposure or age from COPD-specific alterations. The use of a single time point and a defined MOI, selected to balance infection efficiency and epithelial viability, provides only a snapshot of the dynamic host-virus interaction. Moreover, the findings relate specifically to the IAV strain used, and responses may differ with other viral subtypes or co-infection contexts. Finally, in vitro ALI models capture key aspects of airway epithelial biology but do not fully reproduce the complexity of the in vivo airway environment, including immune-epithelial crosstalk. Future work incorporating larger donor cohorts, longitudinal sampling and complementary models will be essential to separate disease-, age- and exposure-related effects and to define the broader relevance of CXCL11-dependent pathways in COPD exacerbations.

In conclusion, we identified CXCL11 as an important COPD-associated secreted mediator involved in the epithelial response to IAV leading to increased mucus production and diminished immune response. Moreover, this report highlights the utility of the ALI model in culturing healthy and COPD epithelial cells to study infection-driven exacerbations. Future studies should explore additional candidate molecules to deepen our understanding and support the development of targeted therapies for COPD.

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