

Cigarette smoke exposure disrupts epithelial barrier function and impairs antiviral immune response to influenza infection

ex vivo

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Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease that affects more than 65 million people worldwide and is predicted to be the third leading cause of death in 2030. Smoking is the major cause for developing COPD. Symptoms include chronic cough, excessive sputum production and breathlessness. Additional occurrence of respiratory viral infections can cause severe exacerbations and are a major cause of death for COPD patients. The mechanisms underlying virus-induced disease exacerbations are not well understood. Therefore, in this study the impact of cigarette smoke exposure on the antiviral immune response of the lower respiratory tract was investigated using H1N1 (California/04/2009) pandemic strain infection of smoke exposed Calu-3 cells and viable human precision-cut lung slices (PCLS).

Methods

Calu-3 cells or human PCLS were cultured at air liquid interface and exposed to high and low dose cigarette smoke for 18 puffs, 35 mL each, using the P.R.I.T ExpoCube[®]. Controls were exposed to clean air. Afterwards, cells or PCLS were inoculated with 5×10^3 ffu/well H1N1 (Calu-3) or 10^4 ffu/well (PCLS) for 1 h and post-incubated for 48 h. The immune response was assessed via analysis of cytokine release by ELISA, MSD, and tissue damage was measured by LDH release. The trans-epithelial electrical resistance (TEER) was measured in Calu-3 cells prior to infection and 48 h after the infection (Fig. 1).

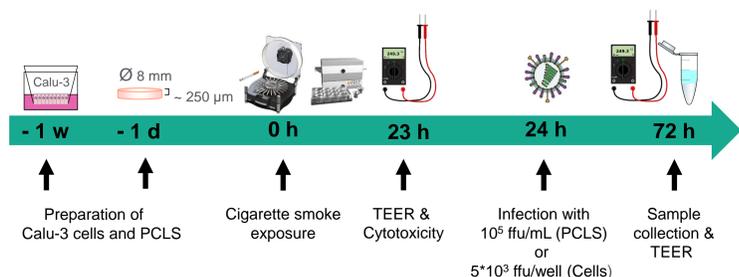


Figure 1. Study design. Human PCLS or Calu-3 cells were exposed to cigarette smoke and 24 h later inoculated with H1N1 for 1 h. Cells and tissue was postincubated for 48 h prior to readout analysis. Localization of virus in PCLS, tissue/cell viability, host immune response and barrier functions were assessed.

Results

Cigarette smoke and H1N1 infection impaired the barrier function of Calu-3 cells

Cigarette smoke exposure dose-dependently induced an increase in LDH release up to 40 % indicating a cytotoxic effect in Calu-3 cells (Figure 2a). In contrast, cigarette smoke induced only a slight cytotoxic effect in a more robust model such as PCLS (Figure 2b).

Furthermore, acute cigarette smoke exposure of Calu-3 cells resulted in a strong concentration-dependent loss of epithelial barrier function as indicated by reduced TEER 24 h after CS exposure (Figure 3a). Additionally, infection with influenza H1N1 significantly lowered the epithelial barrier function in air exposed as well as in smoke exposed Calu-3 cells (Figure 3b).

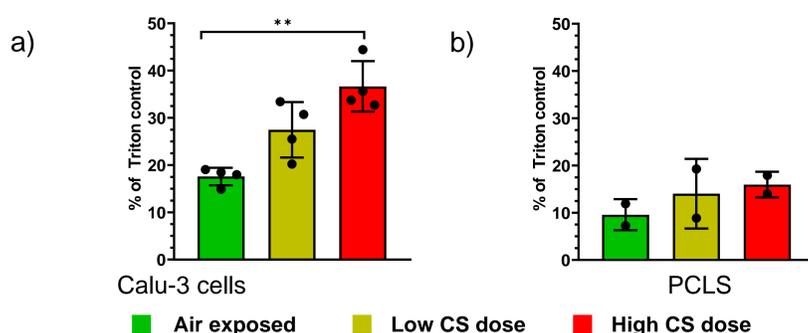


Figure 2. Cigarette smoke exposure led to increased LDH release in Calu-3 cells but not in human PCLS. The increased LDH release was measured 24 h after cigarette smoke exposure by LDH assay. Statistical analysis were done according to a One-Way ANOVA with a Friedman posttest: ** indicates significance with $p \leq 0.01$. Samples were taken from $n=4$ (Calu-3) or $n=2$ (PCLS) independent experiments one with eight technical replicates

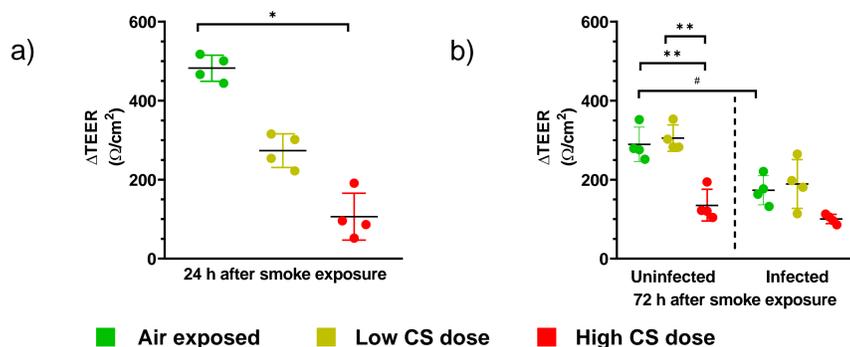


Figure 3. Influenza H1N1 (California/04/2009) infection impaired the cellular barrier function in Calu-3 cells. The TEER value was measured 24 and 72 h after smoke exposure. Data are presented as mean \pm SD from $n=4$ independent experiment with technical quadruplicates. Statically analyses were done according to a One-Way ANOVA with a Friedman posttest (a) or a two-way ANOVA (b): *# indicated significance with $p \leq 0.05$, ** with $p \leq 0.01$. * indicated comparison between uninfected cells, # showed analyses between uninfected air exposed and infected air exposed cells.

Results

H1N1 induced immune response was reduced by cigarette smoke in Calu-3 cells

Influenza H1N1 infection induced an antiviral immune response in Calu-3 cells. The infection led to an increased release of type I interferons and interferon- γ inducible protein 10 (IP-10). The antiviral immune response was reduced if the cells were exposed to cigarette smoke prior to the infection (Figure 4 a-c) Additionally, the induction of pro-inflammatory, pro-apoptotic and chemoattractant cytokines upon influenza H1N1 infection was suppressed by cigarette smoke exposure in Calu-3 cells (Figure 4 d-f).

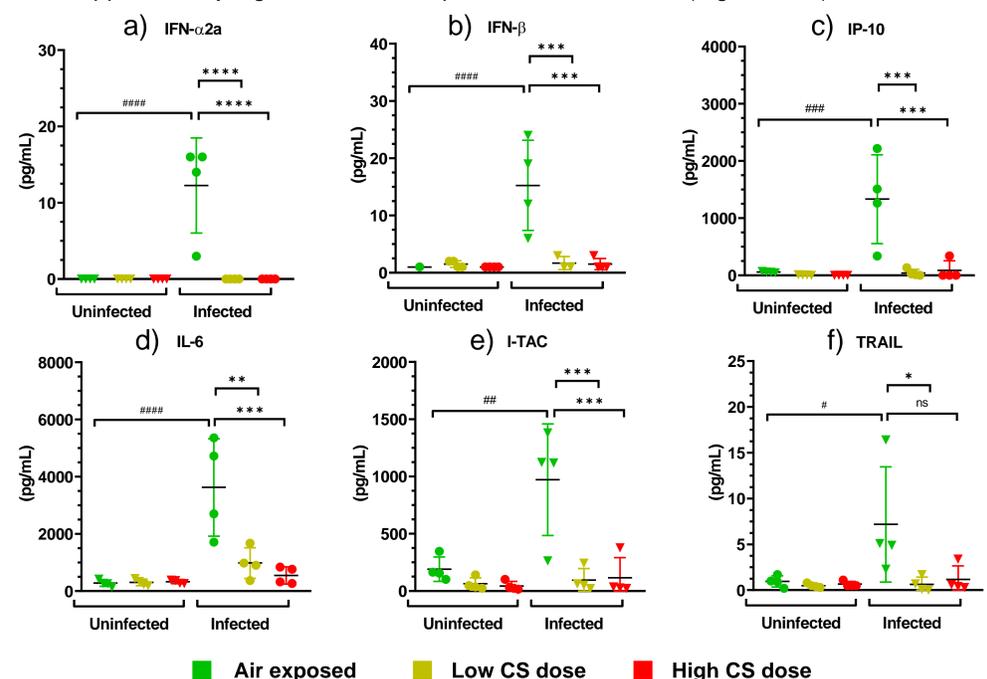


Figure 4. Cytokine release induced by influenza H1N1 was suppressed after cigarette smoke exposure. Calu-3 cells were apically inoculated with influenza H1N1. The cytokine release was determined by MSD-technology and expressed as pg/mL. Data are shown as mean \pm SD from $n = 4$ independent experiments with four technical replicates. Statically analyses were done using a Two-Way ANOVA: **** implies significance with $p \leq 0.0001$. **### indicates significance with $p \leq 0.001$. **/## represents significance with $p \leq 0.01$. */# implies significance with $p \leq 0.05$. * indicates comparison within infected datasets, # shows analysis between uninfected and infected groups.

H1N1 induced immune response in PCLS

Influenza H1N1 also led to an increase of antiviral cytokines in PCLS. The release of type I & II interferons and IP-10 was lowered in smoke exposed influenza H1N1 infected lung tissue *ex vivo* (Figure 5 a-c). The increase of pro-inflammatory and T-cell derived cytokines upon influenza H1N1 infection was diminished by prior cigarette smoke exposure (Figure 5 d-e).

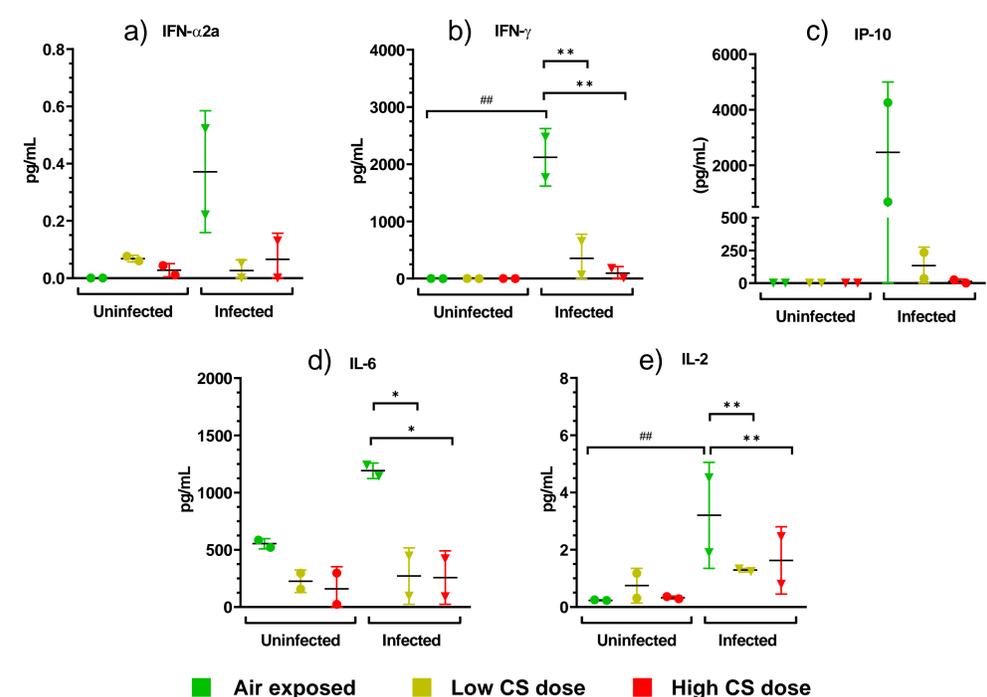


Figure 5. Innate and adaptive immune response upon influenza H1N1 was suppressed after cigarette smoke exposure. PCLS were apically inoculated with influenza H1N1. The cytokine release was determined by MSD-technology and expressed as pg/mL. Data are shown as mean \pm SD from $n = 2$ independent experiments with four technical replicates. Statically analyses were done using a two-way ANOVA: **### indicates significance with $p \leq 0.01$. * implies significance with $p \leq 0.05$. * indicates comparison within infected datasets, # shows analysis between uninfected and infected groups.

Discussion

This study showed that cigarette smoke exposure disrupted the epithelial barrier function in Calu-3 cells. Additionally, the innate and adaptive immune response upon influenza H1N1 infection was suppressed in cells and human lung tissue *ex vivo*. These events might lead to viral persistence and increase the severity of influenza H1N1 infection in smokers or even causing exacerbations in COPD patients.