Club (Clara) Cell 10-kDa protein attenuates inflammation and mucus hypersecretion in bronchial epithelial cells.

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Background

Bacterial lipopolysaccharide (LPS) stimulates IL-8 and granulocyte macrophage-colony stimulating factor (GM-CSF) production in normal human bronchial epithelial (NHBE) cells. Club cell 10-kDa protein (CC10) is an anti-inflammatory protein, produced by epithelial cells, but its role in bronchial airway inflammation is not well established.

Objectives

The aim of this study was to evaluate the effect of CC10 on IL-8, GM-CSF and MUC5AC in NHBE cells stimulated by LPS.

Methods

Cell culture

NHBE cells were differentiated at an air-liquid interface with recombinant human CC10 or vehicle (PBS). Cells were stimulated by LPS and the cell supernatants were harvested on day 14.

IL-8, GM-CSF, MUC5AC secretion

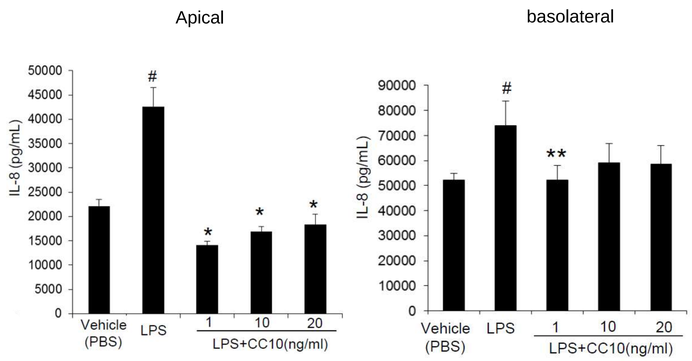
IL-8, GM-CSF, MUC5AC were measured by ELISA. IL-8 and MUC5AC mRNA expression were measured by RT-PCR.

NF-κB and ERK activity

Phosphorylated NF-κB and ERK in whole cell lysates were measured using Western blotting.

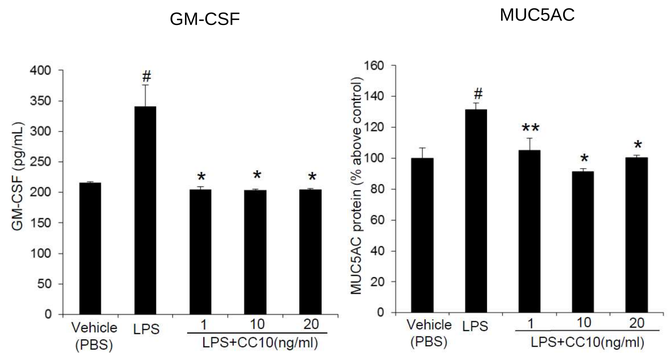
Results

Figure1. CC10 attenuated IL-8 secretion.



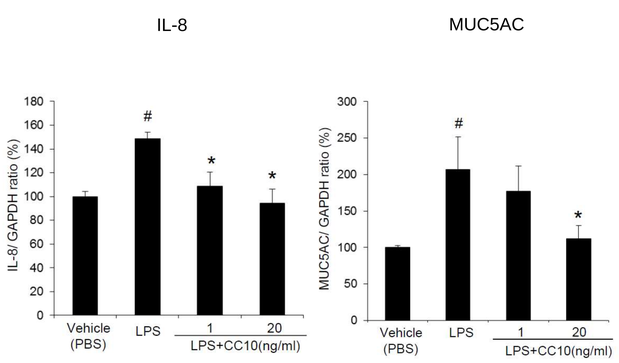
(n=4. #P<0.05, ## P<0.001 compared to LPS vehicle (PBS), *P<0.05, **P<0.001 compared to LPS with PBS.)

Figure2. GM-CSF and MUC5AC secretion were attenuated by CC10.



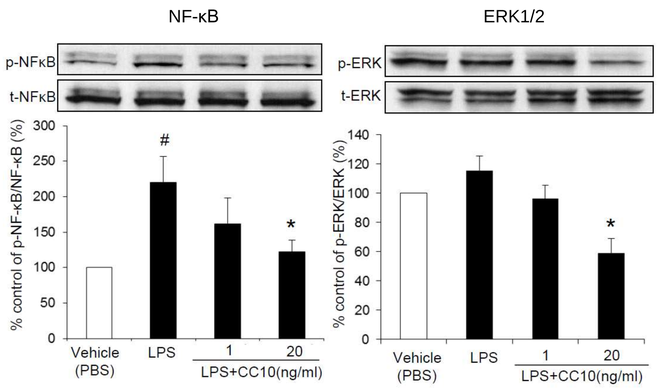
(n=4, #P<0.001 compared to LPS vehicle (PBS), *P<0.05, **P<0.001 compared to LPS with PBS.)

Figure3. CC10 decreased IL-8, and MUC5AC mRNA expression.



(Il-8; n=4, MUC5AC; n=6, #P<0.05 compared to LPS vehicle (PBS), *P<0.05 compared to LPS with PBS.)

Figure4. CC10 inhibited the phosphorylation of NF-κB and ERK1/2.



(n=3. #P<0.05 compared to LPS vehicle (PBS), *P<0.05 compared to LPS with PBS.)

Discussion

CC10 may modulate allergic airway inflammation, and decreased concentrations of CC10 are associated with increased severity of inflammatory airway diseases.

We showed that CC10 attenuated IL-8, GM-CSF and MUC5AC production when cells were exposed to LPS. When CC10 was added to the basal culture media, IL-8 secretion was attenuated in both apical and basolateral media. (Figure1,2)

CC10 blocked LPS-stimulated NF-κB and ERK phosphorylation, and this inhibitory effect of CC10 on cell signaling is consistent with decreased IL-8 and MUC5AC mRNA expression. (Figure 3, 4)

References

1.Hung CH, et al. Regulation of TH2 responses by the pulmonary Clara cell secretory 10-kd protein. *J Allergy Clin Immunol* 114: 664-670, 2004.

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