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## Repeated exposure to hydrogen peroxide enhances TGF- $\beta$ and LPS dependent inflammatory responses in BEAS-2B cells

COPD, Inflammation, Epithelial cell

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Cigarette smoke stimulates an inflammatory response and produces oxidants that cause oxidative stress in the lung, which promotes pathophysiological changes related to chronic obstructive pulmonary disease (COPD) (Kirkham, P. Pharmacol Ther 2006; 111: 476-94). Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is one of the oxidants detected in the breath condensate of COPD patients (Montuschi, P. Clin Chim Acta 2005; 35: 22-34). We aim to understand how chronic exposure to H<sub>2</sub>O<sub>2</sub> alone or in combination with other inflammatory mediators influences epithelial cell responses relevant to COPD lung pathogenesis.

BEAS-2B cells were exposed to H<sub>2</sub>O<sub>2</sub> (2 h/d for 3 days) at different concentrations, alone or in combination with TGF- $\beta$  (10 ng/ml) or LPS (500 ng/ml). Cell viability was assessed. IL-8 and IL-6 were measured by ELISA. Data was analysed using Multiple Comparison Test.

Cells tolerated a repeated exposure of H<sub>2</sub>O<sub>2</sub> (up to 15  $\mu$ M)  $\pm$  TGF- $\beta$  or LPS without significant loss of viability. H<sub>2</sub>O<sub>2</sub> stimulated modest release of IL-8 (mean  $\pm$  SEM; 38 $\pm$ 2 pg/ml) and IL-6 (84 $\pm$ 13 pg/ml). However, repeated 15  $\mu$ M H<sub>2</sub>O<sub>2</sub> exposure significantly enhanced TGF- $\beta$  induced IL-8 (TGF- $\beta$ , 194 $\pm$ 13 vs. TGF- $\beta$ + H<sub>2</sub>O<sub>2</sub>, 279 $\pm$ 10 pg/ml;  $p < 0.0001$ ) but not IL-6 (TGF- $\beta$ , 431 $\pm$ 22 vs. TGF- $\beta$ + H<sub>2</sub>O<sub>2</sub>, 449 $\pm$ 2 pg/ml). H<sub>2</sub>O<sub>2</sub> enhanced LPS secretion of both IL-8 (LPS, 2487 $\pm$ 21 vs. LPS+ H<sub>2</sub>O<sub>2</sub>, 2898 $\pm$ 109 pg/ml;  $p < 0.0001$ ), and IL-6 (LPS, 2469 $\pm$ 72 vs. LPS+ H<sub>2</sub>O<sub>2</sub>, 3277 $\pm$ 62 pg/ml;  $p < 0.0001$ ).

Repeated exposure of BEAS-2B cells to H<sub>2</sub>O<sub>2</sub> induced minimal inflammatory response, but enhanced the effect of TGF- $\beta$  and LPS on cytokine production. These data suggest such combined exposure models may be useful to study the effects of epithelial cell challenge relevant to COPD pathology.