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Cytokine-Induced Glucocorticoid Resistance: Effects Of Full And Partial GR Agonists And Reversal By Formoterol

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INTRODUCTION: Asthma is a chronic inflammatory disease characterised by airways hyperresponsiveness and reversible bronchoconstriction. Inflammatory cells and cytokines are increased in the asthmatic lung and this increase appears to correlate with disease severity. The primary therapy for all but the mildest asthmatics are glucocorticoids, which reduce inflammation by decreasing production of inflammatory mediators, including cytokines, through repression of transcription factors, such as NF-κB, and enhancing the expression of genes (transactivation) that have anti-inflammatory properties. However, in severe asthma responses to glucocorticoids are attenuated. We hypothesized that the generation of inflammatory cytokines in the lung contributes to glucocorticoid resistance in asthma. We therefore investigated whether the cytokines interleukin (IL) 1β or tumour necrosis factor (TNFα) reduce glucocorticoid-induced transcription in human lung cells.

METHODS: Human pulmonary epithelial A549 cells were pre-treated with IL-1β (1 ng/ml) for 2 h before addition of dexamethasone (Dex; 1μM) for either 2 or 4 h. RNA was extracted and subjected to microarray analysis on Affymetrix U95Av2 and B chips. After data normalisation, probe sets were merged for individual genes and then ranked according to Dex-inducibility. Additionally, A549 and bronchial epithelial BEAS-2B cells stably transfected with a 2×glucocorticoid response element (GRE) luciferase reporter, a model of glucocorticoid-inducible gene expression, were pre-treated for 1 h with IL-1β or TNFα respectively, prior to addition of various glucocorticoids. Cells were harvested 6 h after glucocorticoid addition for luciferase assays. Data were expressed as fold and statistics performed using repeated measures ANOVA with Bonferroni's correction for multiple comparisons.

RESULTS: A majority of the genes induced (≥2 fold) by Dex on the microarray were repressed by ≥25% following IL-1β addition. Furthermore, ~25% of these genes were repressed by ≥50%, demonstrating the widespread repressive effects of cytokine treatment. Concentration responses in A549 cells demonstrated that IL-1β pre-treatment resulted in a significant ($p < 0.001$) ~40% decrease in 2×GRE reporter E_{max} values induced by glucocorticoids. Expressed as a percentage of Dex (1 μM) treated, IL-1β pre-treatment reduced the E_{max}s of fluticasone furoate (FF; 0.1 μM), des-ciclesonide (DC; 1 μM) and GW8700086 (GW; 1 μM) from 111±2%, 38±1% and 15±1% to 68±2%, 18±1% and 9±0% respectively, without significantly altering EC_{50} values. TNFα (10 ng/ml) pre-treatment resulted in a similar, significant ($p < 0.001$) decrease in E_{max} values induced in BEAS-2B 2×GRE cells by these and other glucocorticoids. However, when the long acting β_2-adrenoceptor agonist (LABA) formoterol (10 nM) was added together with the glucocorticoid to cells, a full reversal of TNFα-induced decreases in reporter activity was obtained.

CONCLUSIONS: These results demonstrate that a majority of early glucocorticoid-inducible genes are repressed by IL-1β pre-treatment. IL-1β or TNFα pre-treatment reduced 2×GRE reporter activity, irrespective of the intrinsic activity of the glucocorticoid receptor agonist used. This suggests a mechanism whereby increased levels of inflammatory cytokines, such

as TNFα or IL-1β, in the lung during severe asthma reduce responses to glucocorticoids. However, it appears that formoterol functionally reverses cytokine-induced glucocorticoid-resistance. This may, in part, explain the improved clinical benefits of combination therapies comprising a glucocorticoid and a LABA.