Transforming Growth Factor Beta (tgfbeta) Induces Glucocorticoid-Resistance In A549 Adenocarcinoma Cell Line By Reducing Glucocorticoid Receptor Nuclear Localisation

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Rationale: Chronic inflammatory conditions including asthma, show varying levels of resistance to treatment by glucocorticoids (GC). This resistance may involve multiple mechanisms including: oxidative inactivation of histone deacetylase-2, downregulation of the active glucocorticoid receptor-α (GR-α) and an upregulation of the transactivation-inactive glucocorticoid receptor-β (GR-β).

Method: Using A549 human epithelial cell line, GC responsiveness was assessed by measuring dexamethasone (0.1 - 1000nM) regulation of interleukin-1alpha (IL-1alpha) induced interleukin-8 (IL-8) production. Following preincubation of A549 cells in transforming growth factor beta (TGFbeta), we observed a right shift and significant diminution (from control 79 ± 2% to 34 ± 4%) of inhibition in the presence of 400pM TGFbeta (n=7; P<0.05) in the maximum regulatory effect of the GC, Dex on IL-1alpha-induced IL-8 generation. This resistance was observed with TGFbeta concentrations as low as 4pM, and with budesonide as an alternative GC to Dex. TGFbeta-induced resistance to the IL-8 inhibition by Dex was not dependent on TGFbeta trans-differentiation of the epithelial cells to a fibroblast phenotype, as it was observed in pre-incubations of 4 – 24 hours that are too brief to induce epithelial mesenchymal transition (EMT). Treatment of cells with SB431542 1microM, a TGFbeta receptor type I kinase inhibitor, restored the Dex inhibitory effect on IL-8 release (from 17 ± 7% to 45 ± 7%, n=9; P<0.05). Furthermore, in A549 cells transfected with a GC response element (GRE) upstream of secretory alkaline phosphatase (SEAP), it was evident that TGFbeta (40pM) inhibited the GRE response to Dex by more than 80%. Inhibitors of ERK, p38MAPK, Src, PI3K and JNK were investigated for impact on the GRE response. The src inhibitor, PP2 (1 microM) partially restored GRE responses depressed by TGFbeta.

Immunohistochemistry studies showed that Dex-induced GR-alpha translocation in A549 cells pre-treated with 40pM TGFbeta was markedly reduced.

We conclude that TGFbeta is a candidate mediator of GC resistance, acting by reducing nuclear localisation of GR-alpha with a consequent failure to activate transcription of GRE-dependent genes.

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