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Alteration of Toll-like receptor expression in a monocytic cell line following glucocorticoid treatment in two different cell culture conditions

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Introduction: Toll-like receptors (TLRs) are pattern-recognition receptors that are expressed on a variety of immune and non-immune cell types and are responsible for altering the release of inflammatory cytokines upon stimulation. Glucocorticoids are potent anti-inflammatory drugs, which are known to reduce the release of cytokines but their exact mechanism of action has not been fully elucidated. We hypothesized that glucocorticoid drugs may affect TLR expression and thus affect cytokine release.

Materials & Methods: We used the Applied Biosystems TaqMan^{TM} quantitative polymerase chain reaction (QPCR) method to measure (according to manufacturer's instructions) the expression of three TLRs (2, 4 & 5) on the monocytic cell line THP-1 following vehicle (0.1 % dimethyl sulfoxide - DMSO) or treatment with two different glucocorticoids drugs – either dexamethasone (100 nM or 1 uM) or hydrocortisone (100 nM or 1 uM). We used two different culture conditions to test these effects. In the first condition we cultured THP-1 cells in RPMI culture medium and 10% fetal bovine serum (FBS). In the second condition, we used RPMI medium without the phenol red indicator (which has weak affinity for steroid receptors) and used FBS, which was charcoal-stripped (which removes certain growth factors, hormones and cytokines). Each treatment for each culture condition was repeated in five independent experiments. Data were analysed using a one-way ANOVA.

Results: We found that dexamethasone and corticosterone altered the expression of TLR2 and TLR4 on THP-1 cells. This alteration was dependent on the culture conditions of the cells. Both 100 nM ($P < 0.01$) and 1 uM ($P < 0.05$) dexamethasone significantly reduced TLR2 expression in the absence of phenol red and using charcoal stripped FBS. Hydrocortisone had no significant effect on TLR2 expression. Both 100 nM ($P < 0.01$) and 1 uM ($P < 0.01$) dexamethasone as well as 100 nM hydrocortisone ($P < 0.05$) significantly reduced TLR4 expression in the absence of phenol red and using charcoal stripped FBS. The presence of these components in standard culture medium resulted in a significant increase in TLR2 expression following treatment with 1 uM dexamethasone ($P < .05$) as well as a significant increase in TLR4 expression following 1 uM ($P < 0.05$) and 100 nM ($P < 0.01$) dexamethasone. No effect on TLR2 or TLR4 expression was seen with either concentration of hydrocortisone. TLR5 expression was not effected following either drug treatment in either cell culture condition.

Conclusion: Dexamethasone and corticosterone may interfere with inflammation by altering the expression of TLRs, key receptors involved in the release of cytokines. In addition, cell culture conditions may influence the outcome of experiments using drugs that have affinity for steroid receptors.