Evaluation of the mRNA expression response as a novel in vitro tool for assessing sensitivity to steroid treatment

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Glucocorticoids (GC) are the most widely used anti-inflammatory and immunomodulatory drugs. Despite their large clinical impact and justified use, the benefits of these agents are often narrowed by a great inter-individual variability that might potentially lead to treatment failure or drug induced toxicity. A reliable way of predicting response to GC therapy by the patient would therefore be useful in clinical management.

Our aim was to establish an *in vitro* tool for evaluation of the intrinsic sensitivity to GC. For this, we have set up dose-response [^{3}H]-thymidine incorporation assays of methylprednisolone (MP) treatment on inhibition of proliferation of stimulated peripheral blood mononuclear cells (PBMC). In addition, we have developed a means of measuring the action of MP based on its effect on the kinetics of mRNA transcription by the cells. We designed quantitative real-time PCR assays for a panel of mRNA transcripts, the levels of which may be affected downstream of GC treatment. This panel consists of pro-inflammatory mediators (IFNγ, TNF-α, IL- 2, IL-6, IL-8, CCL-2, CCL-3, CCL-5, CCL-13, CXCL-9, CXCL-10, CXCL-11), anti-inflammatory mediators (IL-10, FKBP5, DUSP1, SAP-30, TLR-7) and intracellular signalling molecules (NF-κB, STAT-3, STAT-6, MAPK). Expression levels are standardized to those of reference genes β-actine and GADPH. We have investigated PBMC from healthy donors at the Sanquin Blood bank in Leiden, the Netherlands.

Until now, we have analyzed MP effects on proliferation of phytohaemagglutinin (PHA)-stimulated PBMC in 17 different donors. Non-linear regression of dose–response curves was applied to compute the MP concentration required to reduce proliferation by 50% (IC_{50}). The data showed an high inter-individual variability in MP-IC_{50}, ranging between $1.5x10^{-9}$ and $1.1x10^{-5}$ M. To investigate the mRNA response, we first investigated which cell types in total PBMC fractions are stimulated by either PHA, concanavalin A, CD3/CD28 beads or lipopolysaccharide (LPS). The PBMC were labelled by carboxyfluorescein succinimidyl ester and stimulated for 72h. This was followed by flow cytometric staining for T cells (CD3+CD4+ and CD3+CD8+), B cells (CD19+), monocytes (CD14+), and NK cells (CD3-CD16+CD56+). Results were evaluated as proliferation index number (the sum of the cells in all generations divided by the calculated number of original parent cells). Stimulation with PHA resulted into the highest T cell proliferation index number (1.64) both of T helper cells (CD4+, 1.84) and cytotoxic T cells (CD8+, 1.44), while other cell types did not proliferate. LPS was the only stimulus that induced monocyte proliferation (1.24). Next, we studied in three donors the kinetics of mRNA expression of the different markers at different time periods of stimulation (6h, 12h, 24h, 48h, 72h), using both PHA and LPS. In general, the peak in expression levels was observed at 6 and 12 hours after stimulation. Further experiments will be performed for these two time points, and in combination with different concentrations of MP (10^{-4}M and 10^{-7}M).

In conclusion, we are establishing a novel in vitro tool, based on the mRNA response of different hematopoietic cell types, for quick evaluation of the intrinsic sensitivity to GC treatment. Data from this test will be compared to the outcome of dose-response proliferation assays, and will eventually be placed in the context of clinical outcome in vivo.